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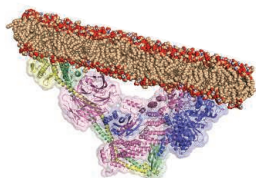
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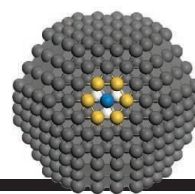
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A chance to get science right

The upcoming Canadian election later this month will provide a welcome opportunity to reboot the federal government's controversial approach to science policy and research. The current Conservative government has been undermining science for the past 9 years, damaging the institutions that make scientific advancement possible and trying to ensure that political and ideological priorities dominate scientific work.

Academic and government research play a particularly important role in Canada, because the private sector does proportionately less research than in most other industrialized countries. As a percentage of gross domestic product, business spending on R&D in the United States is twice as much as it is in Canada. Yet it is public science—in universities and in government—that is eroding under the current government. Science and technology budgets at Canada's 13 science-based federal departments and agencies have been cut by \$596 million (in constant 2007 dollars) between 2008 and 2013. During that period, 2141 full-time equivalent federal scientific positions were eliminated.

Canada's three federal funding agencies for academic research have been hit hard. Allocations for the Social Sciences and Humanities Research Council, the Natural Sciences and Engineering Research Council, and the Canadian Institutes for Health Research have fallen since the present government came to power. The biggest drop has been in the humanities and social sciences, which have seen their real-dollar base funding decline more than 10%. Natural sciences and engineering have experienced a decline of more than 4%, and health sciences funding has dropped by more than 7%. To the money the government does allocate to these agencies, strings are often attached; strings that limit funding to specific political priorities set by the government. The government has also changed membership on the governing councils of these agencies. Historically, they were held primarily by scientific experts in the fields. No longer. On the social sciences and humanities council, the majority of mem-

bers are from the corporate sector, economics, business, and engineering. The council for the natural sciences and engineering has no biologists, chemists, physicists, or mathematicians. Eight of its 17 members are engineers, four are corporate executives, and three are professional administrators. The result is what former University of Toronto president and distinguished medical researcher David Naylor has called a growing emphasis on "fettered"

research: "match-funded, industry-facing research with an applied orientation."

Beyond the funding agencies, there is a good deal of politically motivated defunding. The federal government's hostility to climate science ended support for the Canadian Foundation for Climate and Atmospheric Sciences and for the Experimental Lakes Area, the world's only living natural laboratory for freshwater research. The government also eliminated Canada's mandatory long-form census, the only source of reliable data for much social science research as well as for the development and evaluation of public policy.

The same government has systematically muzzled government scientists, preventing them from responding to media about published articles without political permission. It has transformed Canada's legendary National Research Council, formerly similar to the U.S. national laboratories, into, in the words of Gary Goodyear, then Minister of Science and Technology, a "concierge service" that offers a single phone number to connect businesses to all their R&D needs.

Canada needs to reverse this damage to its scientific enterprise. An immediate priority should be establishing a prominent role for science in government by creating a parliamentary science officer and a parliamentary research and science advisory council composed of top scientists that report directly to Parliament. Hopefully, the widespread and visible public concern about the fall of science will lead whichever party is elected on 19 October to move in a very different direction. It will take concerted action to make this happen.

— James L. Turk



James L. Turk is Distinguished Visiting Professor at Ryerson University, Toronto, Canada; director of the Centre for Free Expression at Ryerson University; and the former executive director of the Canadian Association of University Teachers. E-mail: jim@jameslturk.com



"Canada needs to reverse this damage to its scientific enterprise."

“When my 5-year-old grandson came over and saw the pelvis, he just stood there with his jaw wide open and stared.”

Michigan farmer **James Bristle**, about the 11,700- to 15,000-year-old mammoth carcass unearthed on his property last week.

IN BRIEF

Large mammals thrive post-Chernobyl



Radiation hasn't altered the abundance of elk and other wildlife, scientists say.

Elk, roe deer, wild boars, and other wildlife are thriving in a radiation-contaminated preserve near the Chernobyl nuclear power plant in Ukraine, scientists reported this week in *Current Biology*. They found “no evidence of a negative influence of radiation on mammal abundance” in the Chernobyl exclusion zone, a region largely off limits to people that straddles the Belarus-Ukraine border. Much of the 4200-square-kilometer zone was evacuated after the nuclear plant’s unit 4 reactor exploded in 1986, sending a radioactive plume over Europe. Between 2008 and 2010, Belarusian scientists counted animal snow tracks in the Polesie State Radioecological Reserve in the Belarus sector of the exclusion zone; they had also tallied animal numbers by helicopter in the 10 years after the disaster. The team found no correlation between contamination levels and track counts; instead, they report, mammal populations in the exclusion zone rose after the accident—suggesting that hunting, forestry, and agriculture had suppressed wildlife numbers before 1986. But some scientists argue that the study glosses over evidence that radioactive contamination has damaged individual animals. <http://scim.ag/Chernobylanimals>

AROUND THE WORLD

WHO: End Ebola travel bans

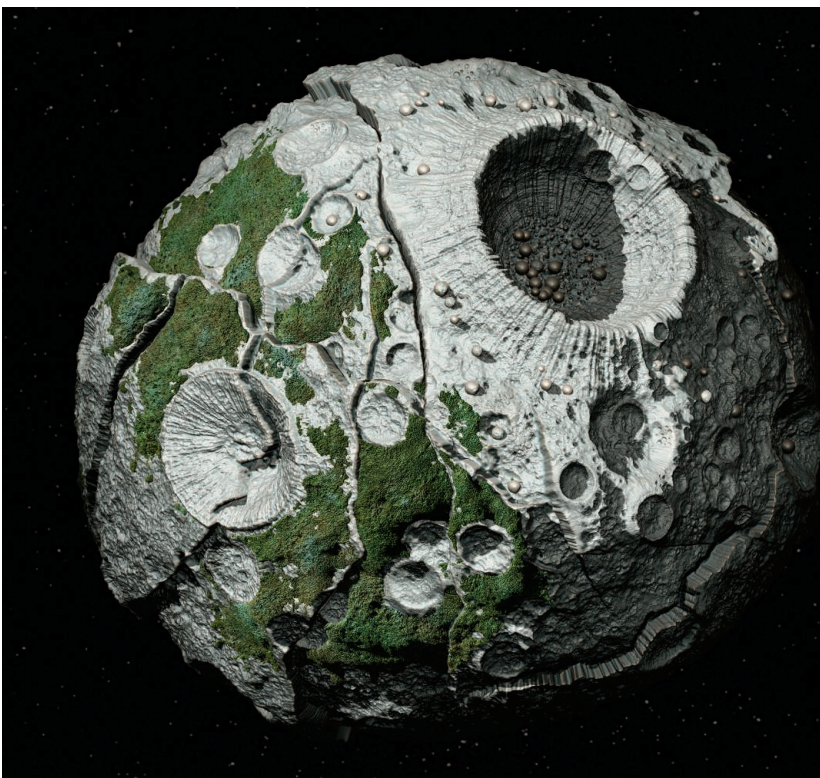
GENEVA, SWITZERLAND | Although the dwindling Ebola outbreak in Guinea and Liberia remains a “public health emergency of international concern,” 34 countries are overreacting to the threat with “excessive or inappropriate travel and transport measures,” announced the World Health Organization (WHO) this week. Overreactions to the threat of Ebola “negatively impact response and recovery efforts,” WHO said in its statement, which followed a meeting of an Ebola emergency committee that oversees International Health Regulations. WHO wants member states to lift travel and trade bans on Ebola-affected countries by the end of the month, but refused *Science*’s request to specify which countries and policies it was criticizing as it is a “dynamic list.” The committee did advise WHO to maintain some temporary restrictions on the two countries with ongoing Ebola transmissions, now at fewer than 10 cases per week; such restrictions include that all travelers leaving these countries should be screened for unexplained fevers.

India’s climate change pledge

NEW DELHI | India, the third largest emitter of greenhouse gases on the planet, announced its long-awaited climate commitment on 2 October, the last major economy to make its pledge ahead of the United Nations talks in Paris this December. The country said it would not place an overall cap on CO₂ emissions, but would instead reduce its “carbon intensity,” cutting emissions per unit of gross domestic product by 33% to 35% in 2030 relative to 2005 levels; emissions would still rise, but more slowly. India also pledged to produce some 40% of its energy from renewable or low-carbon sources by 2030, and vowed to undertake reforestation efforts that would absorb up to 3 billion tons of CO₂ by 2030.

Museum shuttered in budget fight

SPRINGFIELD, ILLINOIS | A political stalemate that has dominated Illinois for



Venus, Psyche missions are among NASA finalists

NASA has announced five finalists for its Discovery program, a low-cost planetary science mission line with a \$500 million cost cap. Selected from among 27 proposals, two of the contenders are missions to Venus—not visited by a NASA spacecraft since 1994—and the other three would study asteroids, including a strange nickel-and-iron asteroid dubbed Psyche (shown). Each finalist will receive up to \$3 million to pursue a more detailed proposal for the final selection about a year from now. Typically, NASA picks just three finalists in its Discovery competitions, which take place every few years. (The inaugural mission was the 1996 launch of NEAR Shoemaker, an asteroid probe.) But this time the agency may choose two winners instead of the usual one, says Michael New, Discovery program scientist at NASA headquarters in Washington, D.C. “It depends on what our budgets in the out years look like,” he says. <http://scim.ag/Discoveryfinalists>

An impact may have stripped the asteroid Psyche of its mantle, exposing olivine-rich rock (green, in an artist's impression) once at its core-mantle boundary.

months has now led to the closure of the iconic 138-year-old Illinois State Museum in Springfield, as well as four satellite museums. The museum, which was home to the largest collection of mastodon fossils in the world, closed its doors on 1 October as the result of a tense budget standoff between the state's Democrat-led General Assembly and its Republican governor. The Illinois Department of Natural Resources, the agency that oversees the museum, announced the pending closure this past June, after the politicians failed to agree on a new budget. Scientists and the public spoke out, writing op-eds to local papers, signing a petition, and

joining a Save the Illinois State Museum Facebook page, but the agency moved forward. <http://scim.ag/ISMclosed>

Trade deal praised, panned

ATLANTA | Public health experts are pleased with some aspects of the Trans-Pacific Partnership (TPP) agreement announced this week, although some are complaining that certain provisions will harm consumers and patients. Pharmaceutical companies have pushed for 12 years of intellectual property protections for biologics, drugs derived from living organisms. Instead, the TPP agreement

offers 5 years of exclusive marketing privileges that prevent competition from generic alternatives. The deal “fell short of Big Pharma's most extreme demands but will contribute to preventable suffering and death,” Peter Maybarduk, an official with the consumer rights group Public Citizen in Washington, D.C., wrote in a statement. Antismoking efforts also won a partial victory. Tobacco companies have used trade agreement clauses to initiate arbitration over plain packaging laws, which they say deprive them of the benefits of their trademarks. The TPP curtails this strategy but does not rule it out entirely. Sharon Friel,

a public health expert at Australian National University in Canberra, worries that tobacco companies could still file dispute resolution claims under the TPP, leading to “regulatory chill,” or a hesitancy on the part of governments to enact tobacco control measures that might invite costly litigation. <http://scim.ag/TPPdeal>

NEWSMAKERS

Brazil reboots science ministry

Brazil's Ministry of Science, Technology and Innovation leadership has changed hands for the third time in less than 2 years, as part of a shakeup announced last week by embattled President Dilma Rousseff to shore up political support in the country's congress. Rousseff named **Celso Pansera**, 52, to replace Aldo Rebelo, who had held the position for just 10 months (*Science*, 16 January, p. 218). Pansera, a first-time federal deputy out of Rio de Janeiro, has no background in science policy or research, but comes from the Brazilian Democratic Movement Party, which controls both houses of congress. Researchers in Brazil have long complained about the science ministry being used as a political bargaining chip; although many researchers had protested the appointment of Rebelo, a reputed climate change doubter, he performed well as minister, says Jacob Palis, president of the Brazilian Academy of



Ground sloth and mastodon bones at the museum.

Sciences. “The scientific community is very disappointed” by the latest reshuffle, says glaciologist Jefferson Simões, of the Federal University of Rio Grande do Sul.

Three Q’s

Iranian President Hassan Rouhani’s cabinet is filled with Ph.D.-trained technocrats. One of the youngest is **Sorena Sattari**, the vice president for science and technology. Sattari, 43, wants to imbue Iran with an “entrepreneurial spirit”; he oversaw the creation of an innovation fund that in 2 years has handed out \$600 million in low-interest loans to 1650 technology start-ups and other firms seeking to branch out in new directions. <http://scim.ag/Sattari>

Q: Iran and Russia plan to jointly develop environmental monitoring satellites. Is this a deepening of scientific ties?

A: We have formed for the first time a joint commission on science and technology cooperation, which is much higher level than our economic joint commission. It’s headed by the deputy prime minister of Russia and myself. For the first time, science and technology is driving the relationship between our countries.

Q: In 2013, President Rouhani pledged to increase academic freedom at Iranian universities. Are conditions improving?

A: It’s unprecedented for an Iranian president to walk in and out of a university and talk to students without some sort of protests. We never experienced this before. It shows how supportive the majority of university students are of his policies. The university atmosphere has become much better compared to the past. Iran is becoming more open.

Q: The Supreme Council of the Cultural Revolution has said that “the revival of the great Islamic civilization” is “contingent upon all-out progress in science.” What does that mean?

A: It means that we want to be the superpower of science and technology in the region. And we want to be No. 1 in the Islamic world as well.

IPCC’s new chief

Hoeseung Lee, a climate and energy economist from South Korea, will be the new chair of the Intergovernmental Panel on Climate Change (IPCC), member governments decided on 6 October at a meeting

in Dubrovnik, Croatia. Lee, currently IPCC’s vice chairman, will succeed Rajendra Pachauri of India, who resigned from his post this year after allegations of sexual harassment (which he denied). Lee has said he wants to “support what has worked, keep what is needed, and change what needs improvement” at IPCC, the world’s main body for synthesizing scientific information on climate change for policymakers.

Scientist sentenced for fake data

Rarely does scientific falsification end in criminal charges, but last week the Copenhagen City Court in Denmark sentenced neuroscientist **Melina Penkowa** to 9 months of “conditional imprisonment” for doctoring data in her 2003 dissertation. The former professor at the University of Copenhagen was convicted of scientific misconduct for fabricating the results of animal research that never actually took place. The court says Penkowa not only made up data for her 2003 thesis, but later fabricated more data to cover her tracks. Penkowa—who pled not guilty—remains free on a suspended sentence. Should she commit academic fraud again, the court says she’ll find herself behind bars.

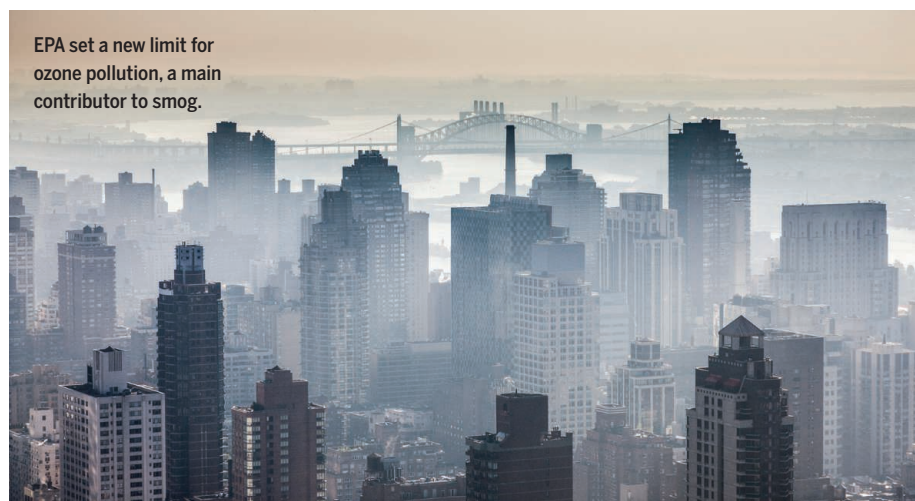
BY THE NUMBERS

37
million

Number of HIV-infected people around the world, up from the current 28 million, who would be eligible for antiretroviral treatment under new World Health Organization (WHO) guidelines. WHO is pushing for early treatment and prevention to end the epidemic by 2030.

\$75
million

Amount of money earned by more than 1000 “predatory publishers,” which charge scientists a fee to publish un-peer-reviewed research in fake journals, in 2014 alone. The average price per article was \$178 (*BMC Medicine*).



EPA set a new limit for ozone pollution, a main contributor to smog.

New ozone limit disappoints all

The U.S. Environmental Protection Agency (EPA) on 1 October tightened its national limits on ground-level ozone pollution, a main contributor to smog. Agency officials say that the target—lowered from the current 75 parts per billion (ppb) to 70 ppb—will lower incidence of asthma and reduce the number of premature deaths. Industry groups, which had waged an expensive and intense campaign to keep the standard at 75 ppb, criticized the decision. The new standard also disappointed environmental advocates, who argue that EPA’s own science advisers have suggested that 70 ppb would provide “little margin of safety” for the protection of public health. But EPA Administrator Gina McCarthy told reporters last week that 70 ppb reflects what the science says about what standard is needed to satisfy the Clean Air Act. <http://scim.ag/ozoneEPA>

IN DEPTH



Protester in a Stephen Harper mask earlier this year dramatizes complaints about “muzzling” of scientists.

a much larger hit than is apparent on paper,” argues Lynne Quarmby, the Green Party’s spokeswoman on science and a molecular biologist at SFU, Burnaby.

To insert such issues into the election, the Professional Institute of the Public Service of Canada, an Ottawa-based union that says its membership includes 15,000 scientists, has produced two radio ads that accuse the Harper government of ignoring scientific advice. And E4D launched a website, True North Smart and Free, which documents dozens of examples of cuts and policies that it says ignore evidence or prevent government scientists from speaking about their work. E4D also is encouraging voters, organizations, and candidates to sign a “science pledge” to publicly state their support for evidence-based policy. And the group sent a questionnaire to each of the main federal parties, asking them to set out their policies on a range of scientific issues.

Those efforts seem to be having some effect. Earlier this month, during one of the five televised debates between the three major party leaders, Trudeau pledged to reinstate the mandatory long-form census. Last week, the NDP’s Mulcair unveiled a science platform that promised to “unmuzzle” government scientists and create a new position, parliamentary science officer, to provide independent advice to legislators. The small Green Party has taken similar stands.

Opposition parties also have promised to reverse cuts to science within government departments, and to reemphasize investment in basic research. And they have promised to break from the Harper regime’s policies on climate change and the environment, which have emphasized fossil fuel extraction and a retreat from tough emissions limits.

Conservative candidates, however, appear unconcerned by the critique. The party has not responded to E4D’s questionnaire, and declined to send a representative to a recent debate on science policy hosted by the University of Victoria. Polls show Canada’s economy remains the dominant issue for voters, and Conservatives believe that signs of improvement have given them an advantage.

Still, many observers expect the final tally to be extremely close, with no party expected to win the 170 seats in Parliament needed to form a government by itself. That could be good news for many scientists: A minority government, even one led by the Conservatives, would likely take more moderate positions on science. ■

Brian Owens is a freelance science writer in St. Stephen, Canada.

SCIENCE POLICY

In Canada, science campaigns for attention from voters

Opponents of Prime Minister Stephen Harper try to make his record on research an issue in election

By **Brian Owens**, in St. Stephen, Canada

Science is making a rare appearance in Canada’s election. As candidates make a last push to Election Day on 19 October, the nation’s leading opposition parties have taken aim at Conservative Prime Minister Stephen Harper’s science policies, which have alienated large segments of the nation’s scientific community.

Science policy isn’t a top concern for most voters in the election, which could send new members of Parliament and a new prime minister to Ottawa. But some research advocates hope the issue could move enough ballots to sway what appears to be a tight three-way race between Harper’s Conservatives, the New Democratic Party (NDP) led by Tom Mulcair, and the Liberal Party led by Justin Trudeau. “Science could be the sleeper issue,” says Kennedy Stewart, the NDP’s spokesman on science issues and a member of Parliament representing a Vancouver district. (Stewart is a political scientist on leave from Simon Fraser University [SFU], Vancouver.)

Traditionally, Canadian elections give short shrift to science. Party leaders didn’t get a single question about science policy at federal election debates between 1968 and

2011, concluded a recent analysis by Evidence for Democracy (E4D), an Ottawa-based non-partisan group founded in 2012 to promote “evidence-based decision-making.” This year, however, research advocates are trying to break that pattern—aided by ongoing controversy surrounding the Harper government’s record on research over the past 9 years (see editorial, p. 139).

Some critics have dubbed it a “war on science.” Since gaining power in 2006, the Conservative government has angered many researchers by eliminating the position of national science advisor and thousands of science-related government jobs, making voluntary a once mandatory long-form census valued by social scientists, ending long-running climate and environmental science programs, and closing research libraries in government departments. Government scientists have been discouraged from speaking to the public and the press. The government has also drawn complaints from backers of basic research by placing a greater emphasis on applied industrial research in many government and university funding programs. Although overall science spending has remained relatively stable in many areas under Harper, “funding for basic research has taken



A boy guides a man blinded by onchocerciasis in Sierra Leone. Ivermectin has prevented more than 500,000 cases of so-called river blindness.

NOBEL PRIZES

Neglected tropical diseases get the limelight in Stockholm

Medicine award honors pioneers who developed powerful drugs against malaria and roundworms

By Erik Stokstad and Gretchen Vogel

If Nobel Prizes were ranked by the number of human lives saved, then this year's laureates for medicine would surely find themselves high on the list. The impact of their research, carried out more than 3 decades ago, continues to prevent death and suffering from parasitic diseases in the developing world.

One half of the prize honored Youyou Tu of the China Academy of Traditional Chinese Medicine in Beijing for her contribution to the discovery of the powerful antimalarial drug artemisinin. The other half was split between William Campbell of Drew University in Madison, New Jersey, and Satoshi Ōmura of Kitasato University in Tokyo, whose work led to a drug that has beaten back river blindness and lymphatic filariasis (often called elephantiasis for the horrifically swollen limbs it can cause).

"I'm in shock," Campbell told *Science* shortly after the announcement on Monday. "I assumed there was no way the prize could be given for this because it was a group effort." (A scientific Nobel can be awarded to at most three people.) But many tropical medicine researchers were elated at the rec-

ognition from Stockholm, which more often awards the prize to basic scientists. "It is extremely rewarding to know that people from the development community have been recognized for work that really helps people," says David Molyneux of the Liverpool School of Tropical Medicine in the United Kingdom.

The discovery of artemisinin emerged from a health crisis in China during the Cultural Revolution of the 1960s and 1970s. Malaria was resurging in North Vietnam, which was then fighting the U.S.-backed South Vietnamese government. The *Plasmodium* parasite was developing resistance to two older drugs, quinine and chloroquine. Under secret orders from Chinese leader Mao Zedong, hundreds of scientists were searching for a cure, but political purges had stifled the work of many. Several scientists turned to traditional Chinese medicine for leads. One was Tu, then a young phytochemist, who noticed that herbal fever remedies often included sweet wormwood (*Artemisia annua*). The Nobel Committee says Tu was the first to demonstrate that an extract from the plant

could cure infected lab animals and humans.

Today, artemisinin and a suite of derivatives are cornerstones of malaria control worldwide. "You can't talk about wonder drugs that often, but artemisinin is one of them," says Jeremy Farrar, director of the Wellcome Trust, a scientific charity in the United Kingdom. The drugs have few side effects and kill malaria parasites quickly, Farrar says, which means they not only save lives of infected people but also slow transmission. Artemisinin was so effective that many Western scientists were initially skeptical, he adds. "There was disbelief that a drug could be this good."

No one disputes artemisinin's impact, but there is controversy over Tu's role in its discovery. When she won a Lasker Award in 2011, some researchers said it was wrong to single her out, as many other Chinese scientists played key roles (<http://scim.ag/LaskerAward>). That debate resurfaced this week. It is "grossly unfair" to recognize Tu alone, says Keith Arnold, a malaria researcher formerly at the Roche Far East Research Foundation in Hong Kong, who was instrumental in bringing artemisinin to Western attention in the 1980s. He notes that some Chinese researchers involved in the project say Tu was neither the first to isolate the active substance, nor the first to show it had anti-malarial properties.

But Yi Rao, dean of the life sciences department at Peking University in Beijing, who studied the early development of artemisinin, says that Tu deserves the recognition. "It is very clear ... that Tu is the most important discoverer and the most

representative of the whole project," he says. "Roles played by others are important, but do not match that of Tu." The quarrel aside, many celebrated the first Chinese woman to win a Nobel Prize, and the first Chinese scientist to win in physiology or medicine. "I think this will stimulate the development of science and technology in China," says George Gao, deputy director-general of the Chinese Center for Disease Control and Prevention. "We need this stimulus."

The award also recognizes "a great contribution of traditional Chinese medicine to the cause of human health," Chinese Premier Li Keqiang told Xinhua News Agency—a sentiment Tu echoed in an interview. But many scientists argue that there is insufficient evidence of efficacy for many herbal

MEDICINE NOBEL

"... for their discoveries concerning a novel therapy against infections caused by roundworm parasites"

William C. Campbell
Satoshi Ōmura

"... for her discoveries concerning a novel therapy against malaria"

Youyou Tu

remedies, and members of the Nobel Assembly, which selects the winners, stressed that Tu used scientific methods: She developed a new way to isolate the active substance and showed that it had potent antimalarial activity in mice and monkeys. "It's very important that we are not giving a prize to traditional medicine," assembly member Hans Forssberg said at the press conference where the prize was announced.

Around the time Tu searched for cures in herbs, Ōmura, a microbiologist, was looking for bacteria that might yield new antimicrobial drugs. From soil samples, he and his colleagues isolated a bacterium, *Streptomyces avermitilis*, which test tube screens suggested might kill parasites. Ōmura's group sent samples to Merck's Sharp and Dohme Research Laboratories in Rahway, New Jersey, where Campbell worked, to be tested as veterinary drugs.

It turned out that they were on to something. In 1979, Campbell and his colleagues reported that *S. avermitilis* could indeed fight parasites and described the active component, ivermectin. Merck chemists built on that work to develop a chemical derivative, dubbed ivermectin, which killed parasitic worms in domestic animals. That led Campbell to suggest that it might be a useful drug for river blindness, or onchocerciasis, a human disease caused by the worm *Onchocerca volvulus*, which is spread by flies and produces larvae that congregate in the eye. In the 1980s, trials in Africa showed that one oral dose of ivermectin a year could keep infections under control. The drug, which has few side effects, was approved for human use in 1987; that same year, Merck pledged to make it freely available to all who needed it for as long as it was needed.

Soon, researchers showed that ivermectin was also effective against the three species of tiny nematodes that cause lymphatic filariasis, and in 1998 the donation program was expanded to include that condition. Both diseases are now on the path to elimination; Molyneux estimates that ivermectin has prevented more than 500,000 cases of blindness.

Looking back, "there was no 'eureka' moment," Campbell recalls. "In that business you learn that what is exciting today is rubbish next week. ... You are always aware that most of these promising candidates fail." Ōmura expressed genuine surprise at receiving science's highest honor. "I've always been proud that my work helped people," he said in an interview on 5 October with the Japanese broadcast network NHK. "I tried to help people. But that is different from being a Nobel laureate." ■

With reporting by Dennis Normile, Kathleen McLaughlin, and Christina Larson.

NOBEL PRIZES

Neutrino oscillations honored

Observations by Japanese and Canadian teams proved ghostly particles can change identity and have mass

By Adrian Cho

Takaaki Kajita of the University of Tokyo and Arthur McDonald of Queen's University in Kingston, Canada, won this year's Nobel Prize in Physics for discovering a weird identity-shifting by ghostly particles called neutrinos. But they might also have been cited for launching a whole new field, as the study of that identity-swapping, or neutrino oscillations, is now perhaps the hottest area of particle physics and could shed light on matters as fundamental as how the universe generated so much more matter than antimatter. The esoteric morphing also has a simple implication: It proves neutrinos have mass.

"It's a strange thing in nature for one thing to change into another," says Alfons Weber, a physicist at the University of Oxford in the United Kingdom. "It's like you throw up an apple and someone catches an orange."

Neutrinos come in three types—electron, muon, and tau—and the new laureates showed that the three can morph into one another as the particles zip along. That proves that neutrinos have mass, as according to Einstein's special relativity, massless particles must travel at light speed, in which

sensitive to muon neutrinos, produced when cosmic rays strike the atmosphere, which occasionally trigger a faint flash of light in the water. In 1998, researchers announced that when they compared the number of muon neutrinos raining down from above with those coming a much longer distance upward through Earth, they found a difference—evidence that the neutrinos were changing identity as they traveled.

McDonald and colleagues confirmed and expanded on the finding, working deep in a mine in Canada at the Sudbury Neutrino Ob-

servatory (SNO), which captures neutrinos in a plastic sphere containing 1000 tonnes of heavy water. They looked at neutrinos from the sun, all of which start out as electron neutrinos. They showed that along the 150-million-kilometer trek to Earth, some of those neutrinos change

into muon and tau neutrinos. The researchers spotted the shift by counting two different types of interactions within their detector, one triggered only by electron neutrinos and the other by all three types of neutrino.

The idea that neutrinos change type had kicked around since 1962, when physicists distinguished electron and muon neutrinos. It got a boost a few years later when scientists first detected electron neutrinos from the sun and found fewer than theory predicted, suggesting that some of them were changing into other types of neutrinos. Still, the concept remained speculative until Super-Kamiokande and SNO weighed in.

Both laureates said they were humbled. "I didn't believe it, and then my knees started wobbling," Kajita said at a press conference at the University of Tokyo. At another press conference, McDonald said, "I have many colleagues who share this prize with me." Each experiment included about 100 researchers.

Now, the science is getting even bigger, as physicists have launched huge experiments that fire neutrinos hundreds of kilometers through Earth to distant detectors. Their goal: to use these almost massless particles to shed light on some of the weightiest questions in physics.

With reporting by Daniel Clery and Dennis Normile.

PHYSICS NOBEL

"... for the discovery of neutrino oscillations, which shows that neutrinos have mass"

Takaaki Kajita

Arthur B. McDonald

"It's like you throw up an apple, and someone catches an orange."

Alfons Weber, University of Oxford, on the unexpected discovery that neutrinos change type

case time for them stands still and change is impossible. Observing "neutrino oscillations" was no mean feat, as neutrinos are nearly impossible to detect. Every second, trillions of them pass through each of us without so much as tickling an atom.

Kajita led researchers working with the Super-Kamiokande, a gargantuan neutrino detector in a zinc mine 250 kilometers northwest of Tokyo that contains 50,000 tonnes of ultrapure water and is lined with 13,000 light detectors. Super-Kamiokande is particularly



PLANT BIOLOGY

How crop-killing witchweed senses its victims

Pinning down molecules key to finding a host plant paves the way for new controls on the parasite

By Elizabeth Pennisi

A field of pink-lavender *Striga* plants can be an enchanting sight. But farmers call this parasitic organism witchweed: It saps the life from rice, corn, millet, sorghum, and other cereal crops, even before its stem breaks the soil surface. In Africa alone, one species, *Striga hermonthica*, causes about \$10 billion in crop losses a year.

Crop developers are trying to identify and breed *Striga*-resistant strains, but the going is slow and more direct countermeasures are desperately needed. This year, in a trio of papers, the most recent on page 203, researchers decipher some of the ways witchweed works its evil magic. They have traced how *Striga* seeds sniff out subtle molecular signals to find new hosts, and how that exquisite sensitivity evolved.

"These papers clearly represent a breakthrough," says Daniel Joel, a plant scientist at Newe Ya'ar Research Center in Ramat Yishai, Israel. With all the recent progress in *Striga* research, "the potential for solving or perhaps ameliorating the problem has changed enormously," adds Michael

Timko, a plant geneticist at the University of Virginia in Charlottesville.

Witchweed likely evolved as a parasite of sorghum in Africa. Today, about 80% of the 30 *Striga* species are found in Africa, mainly in infertile, semiarid tropical regions. *Striga* plagues much of Asia as well, but is less of a problem in Europe, the United States, and China. Each plant can produce 100,000 tiny seeds that may lie dormant in the soil for decades until the right host comes along. Then, the seeds send out a root that penetrates the host's own roots and starts sucking up nutrients. By the time the parasite surfaces and flowers, the serious damage has been done (see diagram, p. 147). Several other plant parasites display a similar uncanny ability to home in on host plants.

An outbreak of *Striga* in the U.S. Carolinas in the 1950s led to an expensive eradication program us-

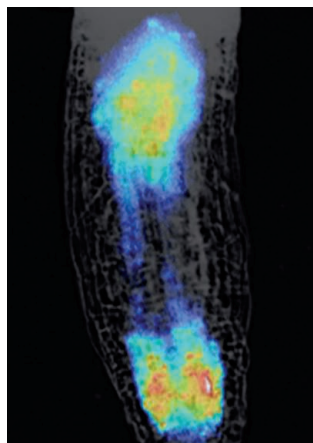
ing ethylene and other chemicals. A burst of subsequent research showed that *Striga* seeds germinate in response to a hormone-like compound, called strigol, released by cotton plants (*Science*, 2 December 1966, p. 1189). Since then, plant scientists have identified more than 16 similar trigger compounds, called strigolactones, made by various host plants.

Their true purpose, of course, is not to lure *Striga*. Recent research has shown that they perform two essential functions: stimulating the growth of soil fungi called mycorrhizae, which form symbiotic connections with plants, exchanging soil nutrients for carbon and nitrogen; and acting as a hormonal signal that spurs the growth of the plant itself.

Striga has hijacked strigolactones for its own purposes, but how it senses these molecules—particularly at the very low concentrations

that plants release into soil—remained a mystery. They are likely in picomolar concentrations in soil, so the strigolactone-sensing molecules, or receptors, used by *Striga* must be several orders of magnitude more sensitive than the host plant's own receptor for these signals.

Witchweed is hard to study, however, because it does not grow well in the lab, and no one knows how to manipulate the parasite's genes to test their function. In the United States and some other countries, *Striga* is



The fluorescence in this germinating *Striga* seed reveals the receptors activated by a host plant's signal.

Despite its colorful flowers, *Striga*, a parasite, can devastate crops, particularly in developing countries.

also classified as a noxious weed, making permission to grow it for research hard to come by. More broadly, the funding and incentives to investigate *Striga* are limited because it is mainly a scourge of poorer countries. “Compared to the scope of the *Striga* problem, its recognition is very low, even [among] plant scientists,” says plant geneticist Yuichiro Tsuchiya from Nagoya University in Japan.

But the discovery that strigolactones also serve as plant hormones has proven a boon for *Striga* science, luring a much broader spectrum of researchers to the study of these signal molecules. This year saw the first international congress on these compounds, and over the past decade publications on them have risen from just a few a year to about 100, says Harro Bouwmeester, a plant physiologist at Wageningen University and Research Centre in the Netherlands.

Tsuchiya and Peter McCourt, a geneticist at the University of Toronto in Canada, are among those swept into the field. While searching for chemicals that stimulate germination in the model plant *Arabidopsis*, they discovered several that resembled strigolactones. After learning what strigolactones did for *Striga*, the pair decided to use *Arabidopsis* to probe how the devastating parasite senses and reacts to the signals.

In *Arabidopsis*, strigolactones break apart when they bind to a receptor called D14. So Tsuchiya’s chemistry colleague Shinya Hagihara and Hagihara’s graduate student, Masahiko Yoshimura, made a synthetic strigolactone that would fluoresce when dismantled, which could help reveal whether *Striga* uses a receptor like D14. When Tsuchiya exposed *Striga* seeds to the mimic, they fluoresced and germinated, he and his colleagues reported in the 21 August issue of *Science*. “So now we had a probe that told us when and where the receptor is active,” in *Striga*, McCourt says.

But the group did not know the actual identity of *Striga*’s germination receptor. At first the researchers thought it might be D14 itself, but in *Arabidopsis*, this receptor controls only branching, not germination—and indeed *Striga*’s version of D14 also does not govern germination. So McCourt, Tsuchiya, and colleagues decided to study a related group of receptors in *Arabidopsis* called HTL, or KAI2, which get seeds to sprout in response to strigolactonelike chemicals. (Forest fires generate these chemicals, and HTL’s

sensing of them seems to help get seeds to sprout quickly in the ash.)

Striga’s genome has 11 HTL genes, and Tsuchiya began testing the ability of each gene’s protein to bind strigolactone in a test tube. With McCourt and his postdoc Shigeo Toh, he also put each *Striga* gene into a line of *Arabidopsis* lacking the single HTL gene it typically harbors. The group found that one of *Striga*’s HTLs triggered *Arabidopsis* seeds to sprout in response to strigolactone, and subsequently they showed that most of the others did as well.

The work dovetailed nicely with research that had recently come out of the lab of

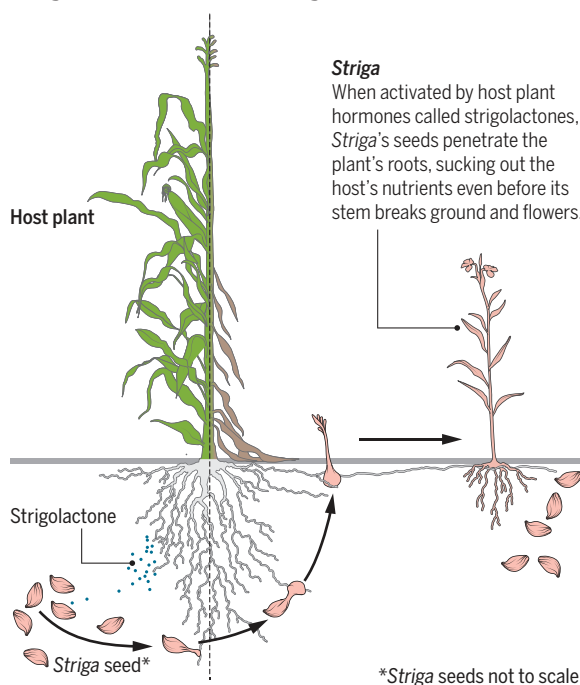
binding pocket compared with that of the apparent original HTL gene. Because that enlarged pocket resembles D14’s strigolactone-sensing one, Conn tested the HTL receptors’ ability to sense the same signal by putting seven HTL genes from *Striga* and a parasitic plant called broomrape into *Arabidopsis*. Some of these genetically altered plants germinated in response to the plant hormone, Nelson’s team reported in the 31 July *Science*, just a few weeks before the publication of the related findings from McCourt and Tsuchiya’s efforts.

Nelson and his colleagues “showed for the first time how [*Striga*’s] strigolactone receptors evolved to support [a] host detection function,” Tsuchiya says. Taken together, the data suggest that a duplication of an ancestral HTL eventually led to two kinds of strigolactone receptors: the D14 that all plants share and use for later growth and the repurposed HTLs that parasites such as *Striga* use to trigger seed germination.

To study the sensitivity of the repurposed receptors, Toh tested transgenic *Arabidopsis* plants equipped with the corresponding *Striga* genes. One, HTL7, made the *Arabidopsis* more than five orders of magnitude more sensitive than normal to strigolactones, Toh, McCourt, and their colleagues report in the study out this week. By crystallizing one of the *Striga* HTL receptors and comparing its structure with that of *Arabidopsis*’s native HTL, they discovered that the parasite’s receptor has twice as big a pocket for binding strigolactones. The size difference, along with differences in the amino acids around that pocket, probably explains the receptor’s extraordinary sensitivity, they note.

With witchweed’s key strigolactone receptors in hand and new tools for studying and pinpointing them, plant biologists are eager to go on attack against the parasite. “These papers tell us what targets to go after,” McCourt says. He envisions improving on a practice called “suicidal germination”: spraying fields with a compound that triggers *Striga* seeds to sprout when there’s no host plant around, so they die. The receptor structure and more details yet to come on which strigolactones are most important to each *Striga* species should point to new weapons, he notes. And by using transgenic *Arabidopsis*, breeders can test whether genetically altering crops to produce different strigolactones could thwart *Striga* activation. Science may yet break witchweed’s evil spell. ■

Wily witchweed’s life cycle



David Nelson, a molecular geneticist at the University of Georgia, Athens. His team had also produced compelling evidence that HTL genes were important for host-sensing in *Striga* and other parasites.

Intrigued by the fact that parasitic plants typically had only one gene for D14 but several for HTLs, Nelson’s graduate student Caitlin Conn had compared the sequences of 53 D14 and 144 HTL receptor genes from 56 parasitic and nonparasitic plants and arranged these genes into a family tree. In addition, she and her colleagues used computer models to predict the 3D structure of each receptor from its gene sequence.

The tree revealed that the parasitic plants had an extra set of HTL genes that were evolving faster than normal. The modeling showed these fast-evolving genes coded for a greatly expanded receptor

BEHAVIORAL GENETICS

Can epigenetics explain homosexuality puzzle?

Study in twin brothers finds link between DNA methylation and sexual orientation

By Michael Balter

Baby, I was born this way,” Lady Gaga sang in a 2011 hit that quickly became a gay anthem. Indeed, over the past 2 decades, researchers have turned up considerable evidence that homosexuality isn’t a lifestyle choice, but is rooted in a person’s biology and at least in part determined by genetics. Yet actual “gay genes” have been elusive.

A new study of male twins, scheduled for presentation at a meeting of the American Society of Human Genetics (ASHG) in Baltimore, Maryland, this week, could help explain that paradox. It finds that epigenetic effects, chemical modifications of the human genome that alter gene activity without changing the DNA sequence, may have a major influence on sexual orientation.

The new work, from Eric Vilain’s lab at the University of California (UC), Los Angeles, is “exciting” and “long overdue,” says William Rice, an evolutionary geneticist at UC Santa Barbara, who proposed in 2012 that epigenetics plays a role in sexual orientation. But Rice and others caution that the research is still preliminary and based on a small sample.

Researchers thought they were hot on the trail of “gay genes” in 1993, when a team led by geneticist Dean Hamer of the National Cancer Institute reported that one or more genes for homosexuality had to reside on Xq28, a large region on the X chromosome. The discovery generated worldwide headlines, but some teams were unable to replicate the findings and the actual genes have not been found—not even by a team that vindicated Hamer’s identification of Xq28 in a sample size 10 times larger than his (*Science*, 21 November 2014, p. 902). Twin studies suggested, moreover, that gene sequences can’t be the full explanation. For example, the identical twin of a gay man, despite having the same genome, only has a 20% to 50% chance of being gay himself.

That’s why some have suggested that epigenetics—instead of or in addition to traditional genetics—might be involved. During

development, chromosomes are subject to chemical changes that don’t affect the nucleotide sequence but can turn genes on or off; the best known example is methylation, in which a methyl group is attached to specific DNA regions. Such “epi-marks” can remain in place for a lifetime, but most are erased when eggs and sperm are produced, so that a fetus starts with a blank slate. Recent studies, however, have shown that some marks are passed on to the next generation (*Science*, 24 January 2014, p. 361).

In a 2012 paper, Rice and his colleagues suggested that such unerased epi-marks might cause homosexuality when they are passed on from father to daughter or from mother to son. Specifically, they argued

immune functions.

To test how important the five regions are, the team divided the discordant twin pairs into two groups. They looked at the associations between specific epi-marks and sexual orientation in one group, then tested how well those results could predict sexual orientation in the second group. They were able to reach almost 70% accuracy, although the presentation makes clear that—in contrast to what a provocative ASHG press release about the study suggested—this predictive ability applies only to the study sample and not to the wider population. Just why identical twins sometimes end up with different methylation patterns isn’t clear. If Rice’s hypothesis is right, their mothers’ epi-marks might have been erased in one son, but not the other; or perhaps neither inherited any marks but one of them picked them up in the womb.

In an earlier review, Ngun and Vilain cited evidence that methylation may be determined by subtle differences in the environment each fetus experiences during gestation, such as their exact locations within the womb and how much of the maternal blood supply each receives.

Such subtle influences are “where the action is,” says psychologist J. Michael Bailey of Northwestern University in Evanston, Illinois. “Discordant [identical] twins comprise the best way to study this.” But he and Rice

caution that the study must be replicated with more twins to be fully credible. Sergey Gavrilets, an evolutionary biologist at the University of Tennessee, Knoxville, and a co-author of Rice’s epigenetics model, adds that the study would also be “more convincing” if the team could link the regions showing epigenetic differences to testosterone sensitivity in the womb.

Vilain’s team stresses that the findings shouldn’t be used to produce tests for homosexuality or a misguided “cure.” Bailey says he’s not worried about such misuse. “We will not have the potential to manipulate sexual orientation anytime soon,” he says. And in any case, he adds, “we should not restrict research on the origins of sexual orientation on the basis of hypothetical or real implications.” ■



A pride march in Belgrade last month. Scientists warn that the new findings should not be used to produce a test or “cure” for homosexuality.

that inherited marks that influence a fetus’s sensitivity to testosterone in the womb might “masculinize” the brains of girls and “feminize” those of boys, leading to same-sex attraction.

Such ideas inspired Tuck Ngun, a postdoc in Vilain’s lab, to study the methylation patterns at 140,000 regions in the DNA of 37 pairs of male identical twins who were discordant—meaning that one was gay and the other straight—and 10 pairs who were both gay. After several rounds of analysis—with the help of a specially developed machine-learning algorithm—the team identified five regions in the genome where the methylation pattern appears very closely linked to sexual orientation. One gene is important for nerve conduction, whereas another has been implicated in



This boy's ethnic group, the Ari, is closely related to a prehistoric African who lived in the Ethiopian highlands.

HUMAN EVOLUTION

Prehistoric Eurasians streamed into Africa, genome shows

First genome of an ancient African suggests widespread mixing with farmers from the Middle East

By Ann Gibbons

Africa is the birthplace of our species and the source of ancient migrations that spanned the globe. But it has missed out on a revolution in understanding human origins: the study of ancient DNA. Although researchers have managed to sequence the genomes of Neandertals from Europe, prehistoric herders from Asia, and Paleoindians from the Americas, Africa's hot and humid climate has left little ancient DNA intact for scientists to extract. As a result, "Africa was left out of the party," says anthropological geneticist Jason Hodgson of Imperial College London.

Until now. A paper published online this week in *Science* (<http://scim.ag/MGLlorente>) reveals the first prehistoric genome from Africa: that of Mota, a hunter-gatherer man who lived 4500 years ago in the highlands of Ethiopia. Named for the cave that held the remains, the Mota genome "is an impressive feat," says Hodgson, who was not involved in the work. It "gives our first glimpse into what an African genome looked like prior to many of the recent population movements." And when compared with the genomes of living Africans, it implies something startling. Africa is usually seen as a source of outward migrations, but the genomes suggest a major migration into Africa by farmers from the Middle East, possibly about 3500 years ago. These farmers' DNA reached deep into the continent, spreading even to groups consid-

ered isolated, such as the Khoisan of South Africa and the pygmies of the Congo.

Anthropologists John and Kathryn Arthur of the University of South Florida, St. Petersburg, discovered the skeleton in 2012 at Mota Cave in southwest Ethiopia after local Gamo elders led the pair to the cave, a hiding place for the Gamo during wartime. The Arthurs unearthed the skeleton of an adult male beneath a stone layer and dated it to 4500 years ago using radiocarbon. The researchers analyzed the petrous bone of the inner ear, which can sometimes preserve more DNA than other bones.

DNA had indeed survived in the ear bone, perhaps aided by the cool temperatures in the highland cave. Researchers were able to sequence each DNA base more than 12.5 times on average, considered a high-quality genome. When population geneticist Andrea Manica and graduate student Marcos Gallego Llorente at the University of Cambridge in the United Kingdom analyzed the sequence, they found that the Mota man had brown eyes and dark skin, as well as three gene variants associated with adaptation to high altitudes; some peaks in the highlands reach 4500 meters, as high as the Matterhorn.

By comparing 250,000 base pairs from Mota's genome with the same sites in indi-

viduals from 40 populations in Africa and 81 populations from Europe and Asia, the team found that Mota was most closely related to the Ari, an ethnic group that still lives nearby in the Ethiopian highlands. They zeroed in on the DNA that the Ari carry but Mota doesn't, which was presumably added during the past 4500 years. They found that Mota lacks about 4% to 7% of the DNA found in the Ari and all other Africans examined. This new DNA most closely matches that of modern Sardinians and a prehistoric farmer who lived in Germany. Hints of these early farmers' DNA previously had turned up in some living Africans, but Mota helped researchers zero in on the farmer's genetic signature in Africa, and to establish when it arrived.

Manica suggests that both the European farmers and living Africans inherited this DNA from the same source—a population in the Middle East, perhaps Anatolia or Mesopotamia. Some of these Middle Easterners headed into Europe and Asia starting 8000 years ago, and were the first farmers of Europe (*Science*, 20 February, p. 814). But other descendants of this population migrated into Africa, likely after Mota lived. This fits with traces of Middle Eastern grains found in Africa and dated to 3000 to 3500 years ago.

Because so many far-flung Africans still carry the farmers' DNA, the study suggests a "huge" migration, Manica says. Farming had already been established in Africa by this time, but the newcomers likely had some advantage that explains why their genes spread. "It must have been lots of people coming in or maybe they had new crops that were very successful," Manica says.

Population geneticist David Reich of Harvard University is struck by the magnitude of the mixing between Africans and Eurasians. He notes that "a profound migration of farmers moving from Mesopotamia to North Africa has long been speculated." But, he says, "a western Eurasian migration into every population they study in Africa—into the Mbuti pygmies and the Khoisan? That's surprising and new."

Migrations into and out of Africa were likely complex and ongoing. "This study is significant on its own," Hodgson says. "But hopefully it is only just the beginning of ancient African genomics." ■





Hostile shores

Migratory bird populations in Asia are crashing as Yellow Sea habitat dwindles

By **Christina Larson**, in Rudong, China;
photography by **Gerrit Vyn**

As dawn breaks over the Yellow Sea, Jimmy Choi wades barefoot through the receding tide, a tripod and powerful telescope on his back. The best time to spot migratory shorebirds is an hour before and after high tide—at 4:10 a.m. on this late summer day—when they cluster on exposed mudflats. Once his tripod is firmly planted in the mud, Choi, an ecology postdoc at the University of Queensland (UQ), St. Lucia, in Australia, peers through his scope at a group of foraging birds. “I’ve got a spoon-billed sandpiper!” he says, triumphant. He checks a GPS device and records the exact location in a waterproof notebook.

Reddish feathers on its neck and upper breast show that the bird is fresh from its breeding grounds, more than 5000 kilometers away on the Chukchi Peninsula of far northeastern Siberia in Russia. Such sightings are increasingly rare. Scientists counted at most 220 breeding pairs of spoon-billed sandpiper at Russian sites in 2009, down from 1000 pairs spotted in 2000 and a huge drop from the estimated 2000 to 2800 of the 1970s, according to a 2010 *Bird Conservation International* paper.

The population collapse has landed the spoon-billed sandpiper on the International Union for Conservation of Nature’s critically endangered list. And it is a sign of serious trouble for many other migratory birds

that sojourn in the coastal wetlands of the Yellow Sea. Of nearly 500 species using the flyway, more than 50 are endangered or vulnerable. The sandpiper “is like the canary in the coal mine—it’s an early warning of what happens when you remove or disturb the habitat that migratory birds depend upon,” Choi says.

Perhaps nowhere else in the world has the loss of habitat for migratory birds been as sudden and severe as in the Yellow Sea. In just 5 decades, between 50% and 80% of the tidal flats along 4000 kilometers of coastline in China and the Koreas has been lost to development, according to an analysis of satellite and other data by Richard Fuller, an ecologist at UQ St. Lucia, and colleagues



Development in China has decimated the wetlands that migratory birds rely on, pushing the iconic spoon-billed sandpiper (above) to the edge of extinction.

published in *Austral Ecology* in January. The loss affects all migratory birds traveling the East Asian-Australasian Flyway. In Australia, at the southern end of the route, “we’ve seen massive declines in all the shorebirds we’ve studied—many species appear to be on their way to extinction,” Fuller says. Nial Moores, an ornithologist and director of Birds Korea in Busan, calls it “one of the greatest ecological disasters on the planet.”

The Rudong wetlands, about 140 kilometers north of Shanghai, are on the front lines. They are “one of the very few healthy mudflats left,” says Jing Li, founder of the Shanghai-based nongovernmental organization (NGO) SBS in China. But already, a 15-meter-high concrete embankment blocks the tides, creating a stark boundary between now-shrunk mudflats and reclaimed land given over to chemical factories and a fish farm pond. Choi and his team, which includes scientists and volunteers from China, Australia, and the United States, are counting and videotaping birds and gathering other data to document the site’s critical ecological importance. And groups including Li’s NGO are promoting public awareness within China, hoping to spur action to save the Rudong and other Yellow Sea habitats. “We want the central government to make policies to stop this degradation,” Li says, such as rethinking the economic incen-

tives pushing local governments to support coastal development.

THE YELLOW SEA has outsized importance because of its location near the center of the East Asian-Australasian Flyway, which spans 22 countries and is used by about 50 million migratory birds. “Think about the map of Asia—really the only places these coastal birds can stop [between the Arctic and lower latitudes] are Japan and along the Yellow Sea,” Fuller says. Those waystations are by far the most threatened part of the flyway. Breeding grounds in Siberia remain intact, and financial incentives to stop bird poaching in wintering grounds in Myanmar and Bangladesh are showing positive, if preliminary, results, Choi says.

In the Yellow Sea, however, development shows no sign of abating. “The drastic loss of habitat [in China, the Koreas, and Japan] is the most important issue,” says Hiroyoshi Higuchi, a professor emeritus at the University of Tokyo who studies bird migratory routes. Japan and the Koreas both have a long history of reclaiming wetlands for agricultural and industrial use. In one big blow to the flyway, in 2006, South Korea erased what Moores calls “one of the most important known shorebird sites in the Yellow Sea”—the Saemangeum estuarine tidal flat—by enclosing it with an enormous seawall.

With much of the shoreline in the Koreas and Japan already heavily developed, scientists are focusing on China. Since the early 1990s, seawalls went from lining just 18% of the country’s 18,000-kilometer coastline to 61%, forming “a new great wall,” researchers from China, the United States, and the Netherlands reported in *Science* last year (21 November 2014, p. 912). They also calculated that reclamation is accelerating. Driven by urbanization and the construction of industrial zones, ports, and other infrastructure, it has surged from 400 square kilometers per year between 2006 and 2010 to a projected 580 square kilometers annually between 2011 and 2020.

Yang Liu, an ecologist at Sun Yat-Sen University, Guangzhou, in China, who studies the nesting survival rate of the Kentish plover, is a witness to the losses. He and his colleagues saw one of their study sites in Bohai Bay, at the northern tip of the Yellow Sea, disappear from one year to the next. “That wetland area was already completely lost,” Yang says.

Upstream development is adding to the toll by cutting off the sediment that rebuilds the coastal wetlands. In the 1950s, the Yangtze River each year added 90 million tons more sediment to Yellow Sea mudflats than was washed away. But during the past 50 years, China has built more than 50,000 dams on

the Yangtze watershed, including the massive Three Gorges Dam, completed in 2003. Now, the sediment balance is negative, with the mudflats losing 60 million tons per year, oceanographer Kehui Xu of Louisiana State University, Baton Rouge, and co-authors calculated in 2010 in *Global and Planetary Change*. Dams along the Yellow River have had a similar effect.

Climate change will also erode habitat, both in the Yellow Sea wetlands and in the birds' northern breeding grounds. Fuller and colleagues project that rising seas will submerge between 23% and 40% of the flyway's intertidal habitats within a century. That could cut populations of 10 long distance migratory bird species by 72%, the researchers concluded in a 2013 study published in the *Proceedings of the Royal Society B*. And

as the tree line moves northward in the Arctic, it will encroach on the tundra that the spoon-billed sandpiper and many other shorebirds prefer for nesting, says Hannah Wauchope, a Ph.D. student of Fuller's who is co-author of a forthcoming paper on climate change impacts on the flyway.

THE SPOON-BILLED SANDPIPER may be among the first victims of habitat loss in the Yellow Sea. Its unusual beak is adapted for very specific foods and tidal environments, Moores says: estuarine sand flats covered with a thin layer of mud and silt. The beak's "wide, spoonlike tip is used for pummeling mud to liquefy it—out of which it extracts very small shrimps." In the past, he adds, "the spoon-billed sandpiper was a top predator in that environment—very effectively

adapted." Now, that specialization could prove its downfall.

Preserving habitat for the spoon-billed sandpiper will benefit all migratory shorebirds, and the impact goes beyond birds. "Wetlands conservation is important for fisheries, for ecosystems services, for providing a buffer against storms, and climate change," Moores says. A bit of evidence was on display that late-summer morning: As Choi's team stomped along in gumboots, a dozen local people with sun hats, buckets, and rakes were following the retreating tide searching for crabs and shellfish.

Fuller notes that China has signed several bilateral agreements with key flyway countries to protect migratory species and wetlands. But for now the economic incentives work against conservation. "Local governments can obtain huge profits from selling the use right of land created by coastal reclamation," Zhijun Ma of Fudan University in Shanghai and his colleagues wrote in their *Science* perspective.

In June, China's State Forestry Administration (SFA), which is responsible for wetlands, and the Chicago, Illinois-based nonprofit Paulson Institute, a think tank founded by former U.S. Treasury Secretary Henry Paulson, launched a new Coastal Wetland Conservation Network in Fuzhou, to share best practices and increase the effectiveness of China's conservation efforts.

"China does not have existing laws or regulations for wetland conservation or protection," Ma Guangren, director of SFA's wetland management center, said in a statement issued during the Fuzhou meeting. "Wetlands

are currently classified as 'unused' land in China and open to development, agriculture, and other uses. Changing this land classification would be a major step forward in improving public awareness of the great importance and value of wetland ecosystem services."

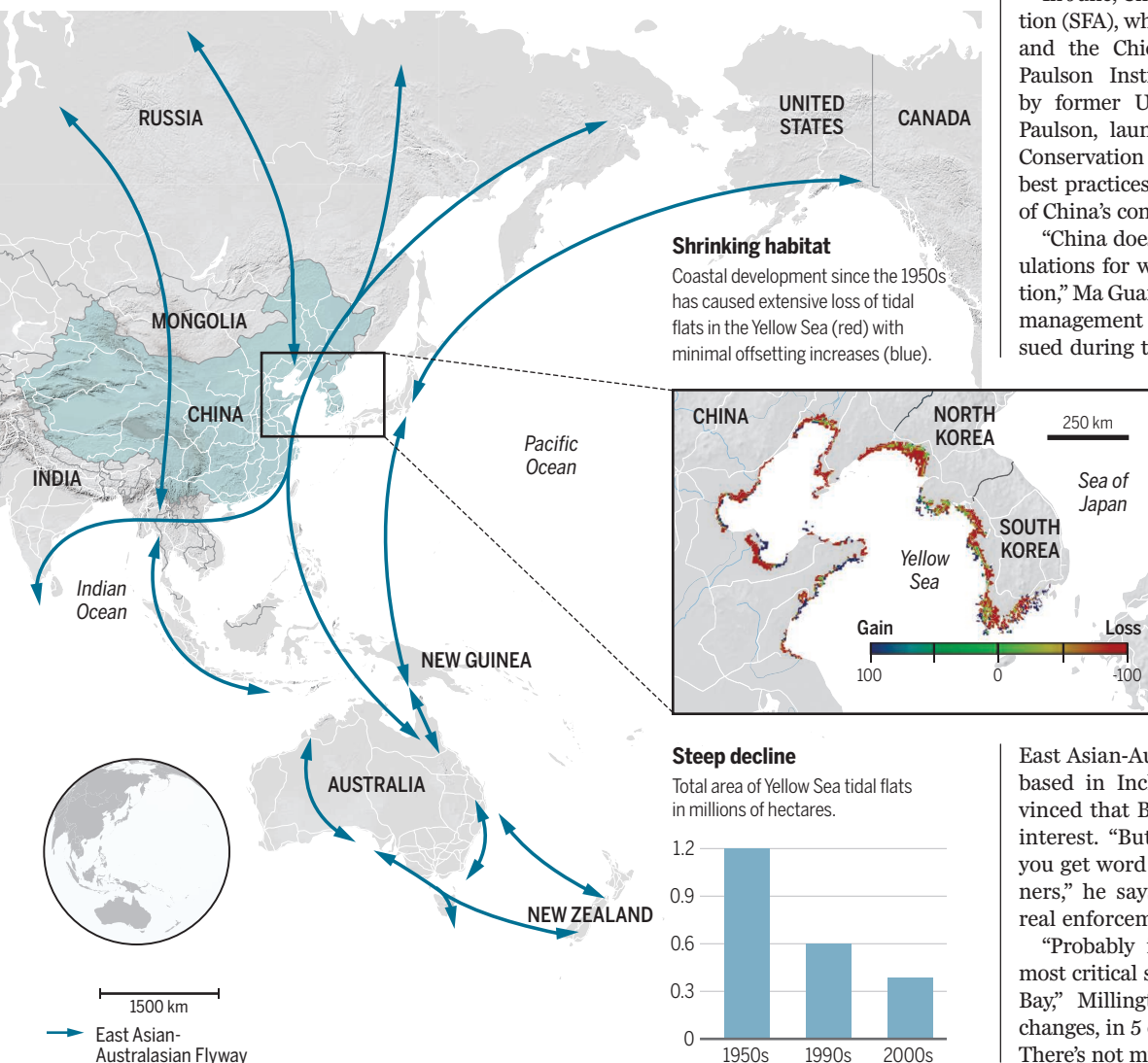
After meetings with SFA officials, Spike Millington, chief executive of the

East Asian-Australasian Flyway Partnership based in Incheon, South Korea, was convinced that Beijing is now taking a greater interest. "But I think the issue is how do you get word out to local government planners," he says, adding there is a need for real enforcement.

"Probably in the whole flyway, the two most critical sites are Rudong and the Bohai Bay," Millington adds. "Unless something changes, in 5 or 10 years, they could be gone. There's not much time." ■

A threatened link

Located near the center of the various routes within the East Asian-Australasian Flyway (blue arrows), the Yellow Sea is a critical way station for more than 50 million migratory birds representing nearly 500 species.



Feeling the chill

The bizarre properties of liquid helium transfixed generations of physicists. But its spell is fading *By Adrian Cho*

The physics meeting was meant to feel like one of those motivational seminars where attendees get fired up by talks from former politicians, sports stars, or astronauts. Convened at the behest of the U.S. National Science Foundation (NSF), the 3-day workshop at the State University of New York at Buffalo aimed to inspire younger physicists to study quantum fluids—essentially liquid helium, one of the weirdest substances on Earth and, for more than a century, a scientific gold mine. In the final session, four speakers, including three Nobel laureates, summarized the grand challenges for the field.

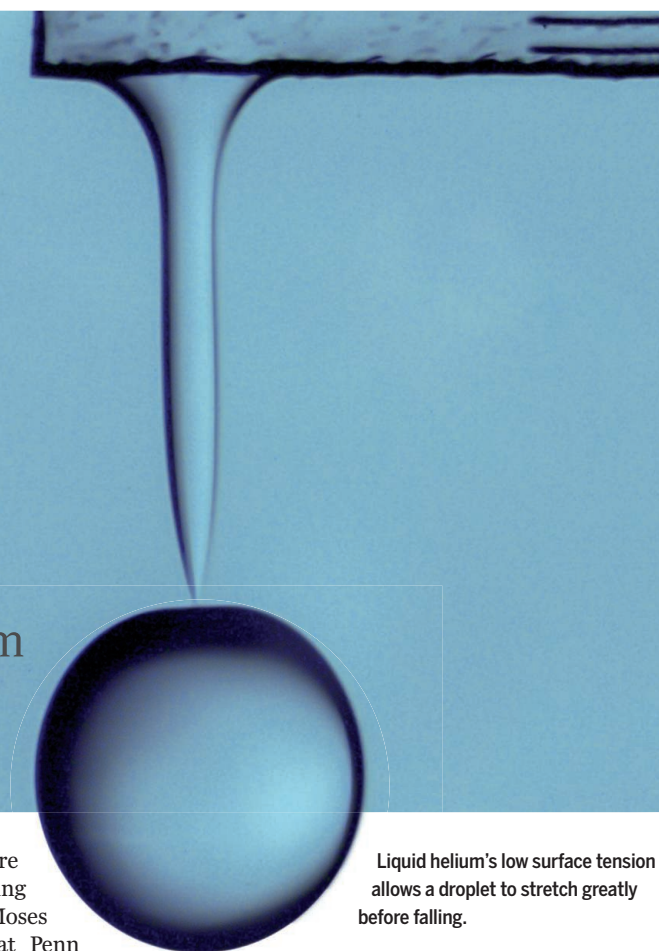
Yet the event seemed to inspire more anxiety than enthusiasm. “If a graduate student comes and hears about these phases of helium, he really doesn’t understand it,” Pavlo Bulanchuk, a graduate student at Pennsylvania State University (Penn State), University Park, said during the group discussion that closed the August meeting. “Why would a graduate student go into this?”

Like a needle to a boil, the question cut to the problem the meeting aimed to address: Once perhaps the most illustrious branch of condensed matter physics, the study of liquid helium is ailing. Especially in the United States, the field is contracting, as older physicists retire and few younger ones enter the field. Even submissions to the field’s main journal, the *Journal of Low Temperature*

Physics, are falling. “We’re afraid the field is going to die with us,” says Moses Chan, 68, a physicist at Penn State who organized the workshop.

Studies of liquid helium, the only substance that won’t freeze at absolute zero temperature, have bagged five Nobel Prizes—one more than superconductivity, the other star of condensed matter physics. The liquid displays bizarre quantum mechanical properties such as “superfluidity”—the strange ability to flow without resistance—and has been the nexus between fundamental theory and condensed matter physics. Liquid helium’s advocates think it still has much to offer. “I’m hoping to identify the scientific issues for the community and use this as a hook to draw people into the field,” says Paul Sokol, NSF’s program manager for condensed matter physics.

But with much already known, breakthroughs are hard to come by. And as the recondite talks that nonplussed Bulanchuk suggest, the field has become insular, defined by narrow questions and exotic, challenging experimental techniques. “The helium community is quite closed,” says Tin-Lun “Jason” Ho, a theorist at Ohio State University (OSU), Columbus. “They have not learned to go out and really integrate with other fields.” The challenge may be to make helium relevant to the rest of physics again.



Liquid helium’s low surface tension allows a droplet to stretch greatly before falling.

LIQUID HELIUM proved a scientific boon almost as soon as Dutch physicist Heike Kamerlingh Onnes produced it in 1908, chilling the gas with liquid nitrogen and liquid hydrogen and cycling it through a constriction to make it expand and cool further. At 4°C above absolute zero, or 4 K, it finally liquefied. Three years later he used the liquid to chill mercury and found that at such temperatures the metal conducts electricity without resistance—the first observation of superconductivity. In 1913, Kamerlingh Onnes won a Nobel for liquefying helium and all it led to.

Helium also displays wondrous behaviors of its own because of two properties of its atoms. They are so inert they won’t bond even to themselves, which is why liquid helium won’t freeze unless squeezed to 25 times atmospheric pressure. The atoms are also so light that they jiggle wildly, even at close to absolute zero. That motion broadens each atom’s quantum wave, which gives the probability of finding it in one place or another. In liquid helium, those waves can overlap and form a single macroscopic quantum wave—the root of the liquid’s bizarre properties.

The main one, superfluidity, was discovered in 1937 by Russian physicist Pyotr Kapitsa and, independently, Canadians John Allen and Donald Misener. They found that

below 2.2 K, one of helium's two isotopes, helium-4, flows without resistance, enabling it to zip through holes impassable to any other liquid. The lighter isotope, helium-3, forms a superfluid even closer to absolute zero, below 3 millikelvin, as physicists at Cornell University discovered in 1972.

The rules of quantum mechanics help explain the difference. Helium-4 atoms have zero spin, which means they belong to the class of particles called bosons. According to quantum mechanics, any number of identical bosons can crowd en masse into a system's single lowest energy quantum wave (see figure, below). That process, called Bose-Einstein condensation, lies at the heart of superfluidity in helium-4.

Helium-3 atoms don't fall into lockstep as

whole like water in a bucket, but will sprout quantized whirlpools known as vortices. Scoop up a ladle of the stuff and it will spontaneously creep over the edge of the ladle to drop back down into the original container to lower its energy. Even though it's a liquid, in its superfluid state helium is perfectly orderly and has no entropy.

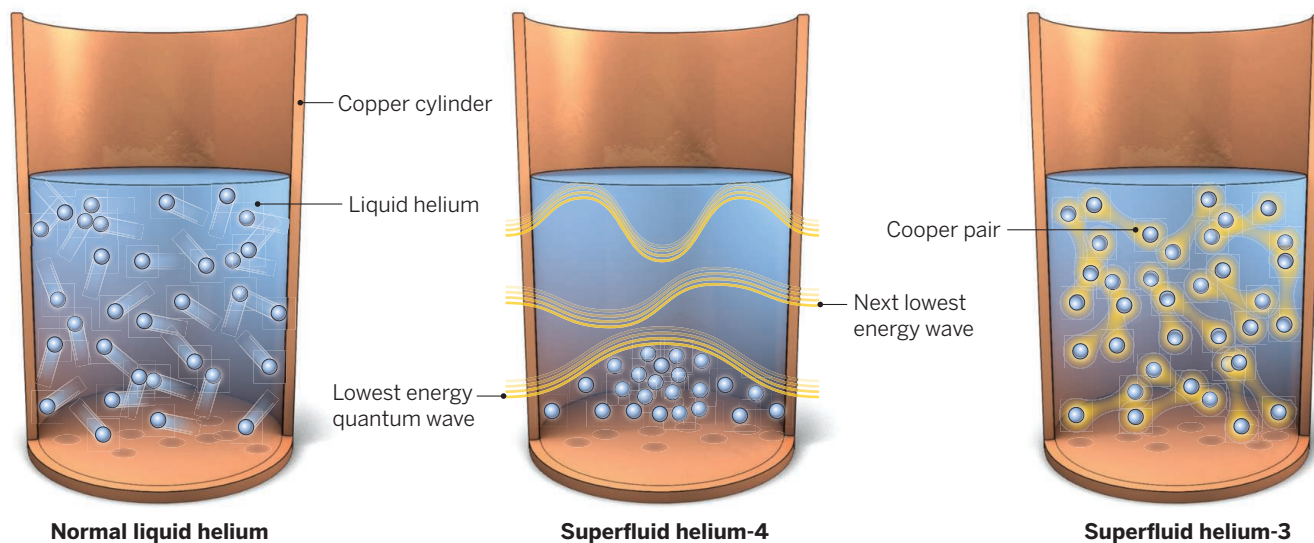
Over the decades, researchers have developed a battery of distinctive mechanical and thermal techniques to probe its behavior. They monitor the onset of superfluidity, for example, by setting a small can of liquid helium twisting atop a stiff shaft and lowering its temperature. A speedup in the infinitesimal twisting reveals that the helium has let go of the walls of the can—a sign that friction has vanished. Researchers have studied

pulsars. Schwab and his student, Laura De Lorenzo, plan to use microwaves to siphon energy out of a fist-sized canister of the superfluid until it reaches the state of least possible motion—the quantum ground state. In that state, the slightest jiggle, including ones as subtle as gravitational waves, should set it ringing with sound waves like an organ pipe, Schwab says.

Others are turning new tools on helium itself. For example, in the past decade researchers have developed techniques to visualize vortices, which can form orderly patterns or tangle in quantum turbulence. Now, Andrey Vilesov, a chemical physicist at the University of Southern California in Los Angeles, has taken those efforts to the extreme, blasting droplets of superfluid helium-4 with

Catch the wave

The presence of a macroscopic quantum wave gives superfluid helium bizarre properties. In a normal liquid, atoms jumble together. In superfluid helium-4, atoms crowd into the same quantum wave, and in superfluid helium-3 atoms pair and the pairs form a quantum wave.



easily. They have half a unit of spin, which means they are fermions, and no two identical fermions can occupy the exact same quantum state. So helium-3 takes a different route to superfluidity: Its atoms form loose alliances called Cooper pairs, which then weave together in a single quantum wave. A similar effect binds electrons (also fermions) in a superconductor, enabling them to flow without resistance. But the waltz of paired atoms in helium-3 is more complicated, so bulk helium-3 has two superfluid phases, A and B, described by quantum waves with different symmetries. They form at different temperatures and pressures and can be distinguished with magnetic measurements.

However it forms, the quantum wave in superfluid helium has weird consequences. Set the liquid moving and it can flow forever. Try to rotate it and it won't turn as a

helium-3 and helium-4 in mixtures, in films, in the random holes of porous glasses—in just about every condition experimental ingenuity permits.

NOW, HELIUM PHYSICISTS FEAR they have become victims of their own success. “We have an absolutely quantitative understanding of this superfluid state,” says Yoonseok Lee of the University of Florida in Gainesville. “That shouldn’t be held against us.”

At the Buffalo workshop, physicists had lots of ideas for building on the past decades’ accomplishments. For example, Keith Schwab of the California Institute of Technology (Caltech) in Pasadena aims to use a small volume of helium-4 to build a detector for gravitational waves—ripples in spacetime set off by stellar objects such as wobbling

the enormous x-ray laser at SLAC National Accelerator Laboratory in Menlo Park, California, to image for the first time the sub-nanometer-size cores of the vortices.

The most promising general trend is the merger of nanotechnology and helium-3, physicists say. For example, John Saunders of Royal Holloway, University of London in Egham, U.K., and colleagues are studying the behavior of helium-3 squeezed into nanometer-scale cavities, which change the conditions at which the A and B phases form. Such confinement could even produce new superfluid phases and quantum waves.

Such techniques could pay off at the conceptual frontier in helium physics: so-called topological states, which put a twist on condensed matter theory. In a superconductor, when the quantum wave describing the electrons is mapped in a certain way, it can wind

on itself like a Möbius strip—the one-sided shape made by joining the ends of a twisted strip of paper. The winding gives the wave a “topological charge” that it must shed at the material’s surface. That esoteric unwinding has concrete consequences: On the surface, unquenchable currents must flow.

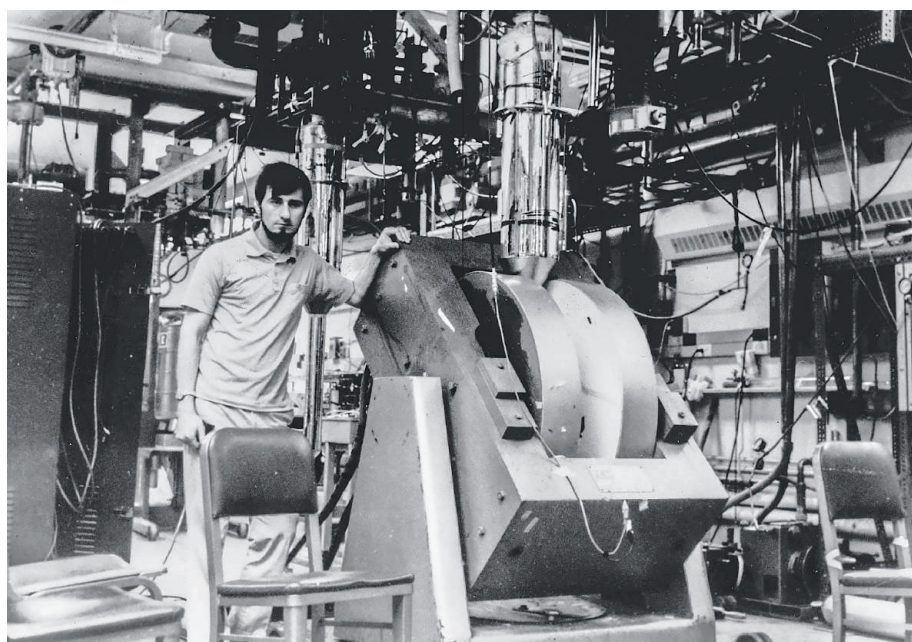
Topological states are the hottest thing in condensed matter physics, for both their exotic qualities and their practical potential. The surface currents can be thought of as composed of “quasiparticles” that could be their own antiparticles—and could also provide a medium for quantum computations. Experimenters are still searching for a topological superconductor. (Strontium ruthenate may be one.) But helium physicists say they already have one: superfluid helium-3. “In the electronic system, they’re still searching for materials that exhibit these effects, but in helium we already have it,” Florida’s Lee says.

Nanoscale experiments could be key to spotting the surface currents in helium. If helium-3 is confined to a tiny slab, the A phase should produce an unquenchable flow of atoms around the slab’s edge, making it act like a gyroscope, an effect that Séamus Davis of Cornell University aims to detect. In the B phase, the current should consist of atoms spinning in opposite directions, flowing in opposite ways. To detect that minuscule “spin current,” Royal Holloway’s Saunders plans to use ultrasensitive nuclear magnetic resonance techniques.

IN SPITE OF THESE OPPORTUNITIES, the field is contracting. For example, Florida had four professors working on helium, Lee says, and now it has two. Meanwhile, only a handful of younger physicists have landed faculty positions in the field. Those trends may reflect problems besides finding the next big thing.

For one, when it comes to studying macroscopic quantum effects, helium physicists no longer have the field to themselves. In 1995, atomic physicists used laser light and magnetic fields to cool gases of bosonic atoms such as rubidium-87 to nanokelvin temperatures, achieving Bose-Einstein condensation. Eight years later, they produced Cooper pairing with fermionic atoms such as potassium-40. Such quantum gases display many of the same behaviors as superfluid helium.

The cold gases have their limitations, notes Wolfgang Ketterle of the Massachusetts Institute of Technology in Cambridge, who shared the Nobel Prize in 2001 for the discovery of Bose-Einstein condensates. “We cannot attach thermometers, wires, and transducers to our samples,” he says. But others note that the cold atoms are easier to manipulate than superfluid helium.



Douglas Osheroff was a graduate student at Cornell University in 1972 when he and two colleagues discovered superfluid helium-3. The advance marked the heyday of the study of liquid helium.

In fact, low-temperature physicists often pride themselves on their ability to do very hard but old-fashioned experiments, Caltech’s Schwab says. That tradition can be a barrier to new talent. “Students see that and they don’t want it,” he says. “They’d rather go to a quantum optics group that’s doing amazing stuff.”

The challenging lab techniques also give pause to university hiring committees, says John Davis, a younger physicist at the University of Alberta, Edmonton, in Canada, who has devised a nano-mechanical whistle that he hopes to use to detect topological states in helium-3 by their effect on how helium flows through it. “You hire somebody who has one big experiment and it won’t be done for 7 years and that person could get scooped anyway,” he says.

Perhaps most important, some researchers say, the field has grown inward looking, focusing on problems in which the broader physics community takes little interest, as the Buffalo workshop itself showed. It aimed to identify grand themes and future opportunities, but often seemed like just another business-as-usual conference. “I thought that 60% of the talks were a complete waste of time,” says George Pickett of Lancaster University in the United Kingdom.

For example, some physicists are still talking about supersolidity—a phenomenon that captivated researchers a decade

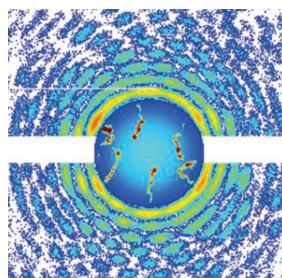
ago but didn’t pan out. In 2004, Penn State’s Chan and Eunseong Kim, now at the Korea Advanced Institute of Science and Technology in Daejeon, reported that below 200 millikelvin, crystalline helium-4 appeared to flow like a superfluid. Some theorists said that was impossible in a perfect crystal, and 3 years ago Chan himself showed that the signal was due to a stiffening of the solid and not to flow (*Science*, 5 October 2012, p. 25).

Still, some signs of flow in solid helium-4 remained, albeit only in crystals riddled with defects and adulterated with helium-3, and physicists have continued to study it. Ho, the theorist from OSU, questions that effort. “If it’s not what Kim and Chan reported, then why do we still have to talk about it?” he asks.

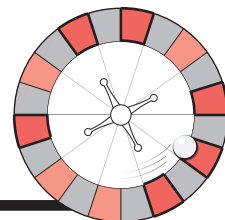
Yet one big discovery would revive the field, Ho says. James Sauls, a theorist

at Northwestern University in Evanston, Illinois, agrees. “I know there’s exciting physics to be done in this field,” he says, “I have no doubt about it.”

Alberta’s Davis, who works with helium only part time, is less sanguine. Helium physics will live on, he predicts, but as a niche endeavor. “It will be a smaller field, probably, and people will be doing it as part of their program and not the whole thing,” Davis says. “That’s a bit depressing, but maybe that’s the nature of the beast.” ■



X-rays reveal tornadolike vortices swirling in a droplet of helium-4.



PERSPECTIVES

PLANT SCIENCE

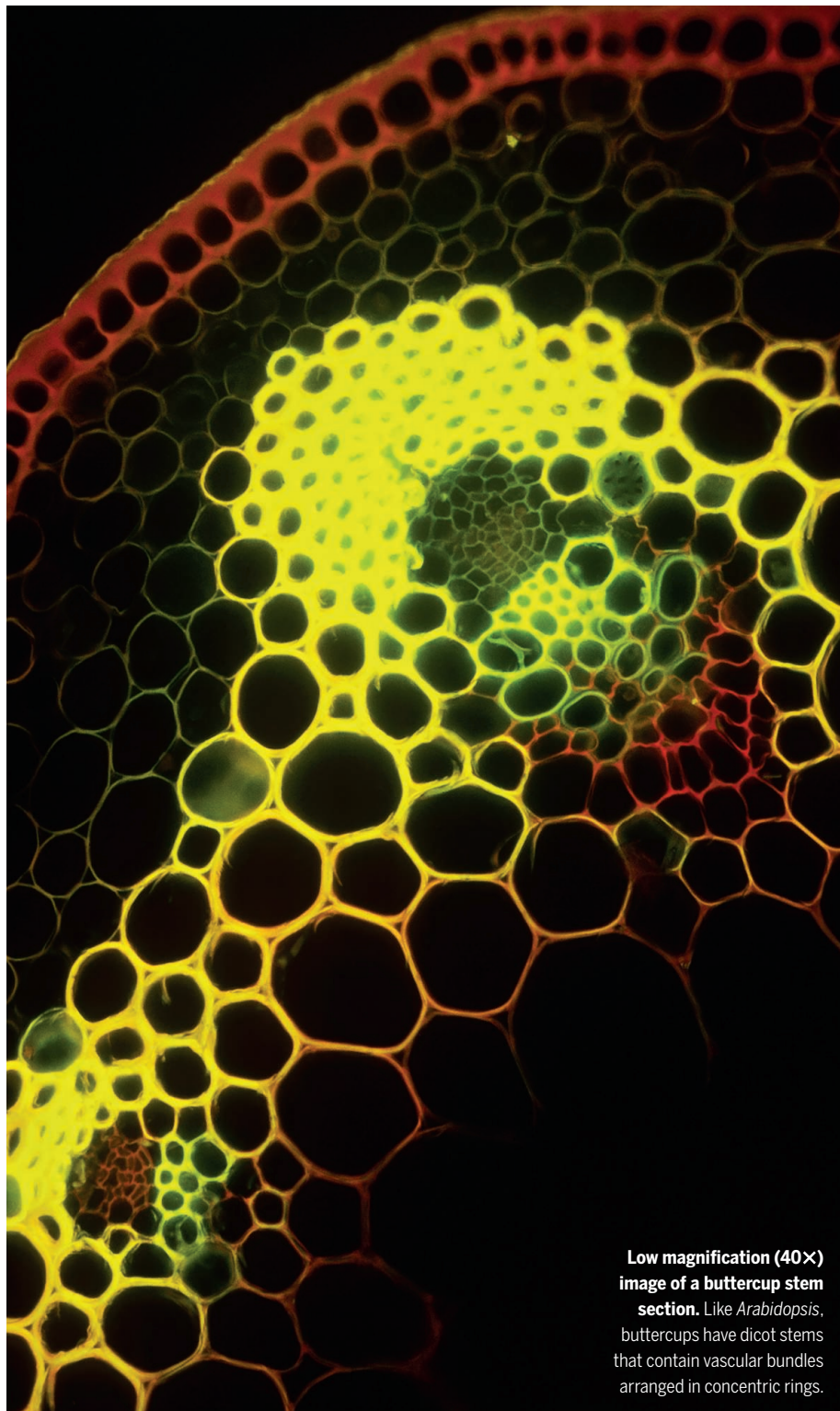
Another brick in the wall

An inducible secondary wall system reveals how most plant biomass is synthesized

By **Rene Schneider**¹ and **Staffan Persson**²

Plant biomass is essential for humans and animals and is of great importance as a raw material for many industries, including food, feed, biomaterial, and fuel (see the image). The bulk of a plant's biomass resides in a carbohydrate-enriched extracellular matrix that surrounds every plant cell. Growing plant cells are encased in an elastic primary cell wall that dictates the direction of cell expansion (see the first figure, panel A). A durable and rigid secondary wall is deposited around cells that need extra support for their function, such as water-transporting vessels and fiber cells in plant stems (see the first figure, panel B). On page 198 of this issue, Watanabe *et al.* (1) provide a comprehensive cell biology study of how secondary wall cellulose is synthesized in plants.

Cellulose, the most abundant biopolymer on Earth, is a key component of both primary and secondary cell walls (2). It consists of β -1,4-glucan-linked chains that coalesce via hydrogen bonding to form paracrystalline microfibrils (3). The tensile strength of these microfibrils is on par with that of steel, and the direction of the microfibrils in the cell wall thus directs cell growth and hence shape. The production of cellulose microfibrils has been visualized during primary wall synthesis (4), but similar observations



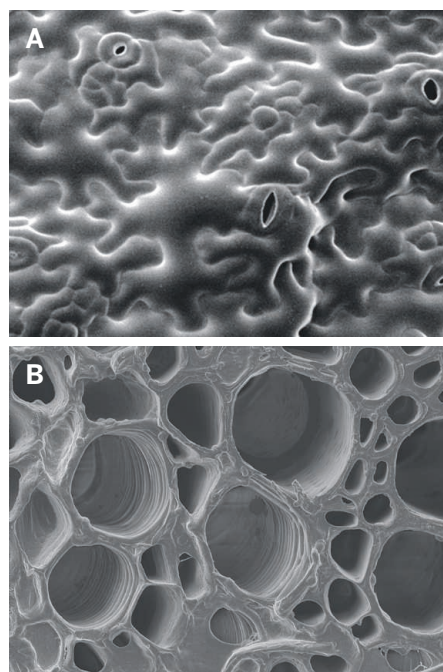
Low magnification (40 $\times$) image of a buttercup stem section. Like *Arabidopsis*, buttercups have dicot stems that contain vascular bundles arranged in concentric rings.

¹Max-Planck-Institute for Molecular Plant Physiology, Am Muehlenberg 1, 14476 Potsdam, Germany. ²ARC Centre of Excellence in Plant Cell Walls, School of Biosciences, University of Melbourne, Parkville 3010, VIC, Melbourne, Australia. E-mail: staffan.persson@unimelb.edu.au

of secondary wall cellulose deposition have remained challenging.

Cellulose microfibrils are synthesized at the plasma membrane by cellulose synthase complexes (CSCs) (5). During the initial phases of cellulose synthesis, the nascent cellulose microfibrils are thought to become entangled in the cell wall; further synthesis then causes the CSCs to migrate through the plasma membrane. Support for this hypothesis comes from the crystal structure of the catalytic subunit of bacterial cellulose synthase, which translocates the growing glucan chains through a pore (6). The notion that the plasma membrane-based CSCs are motile was corroborated by the use of fluorescently tagged primary wall cellulose synthase proteins (4). These primary wall CSCs move with a speed of ~250 nm/min (see the second figure, panel A). If the speed corresponds to the catalytic activity, then this is equivalent to the addition of ~500 glucose units per minute to a growing cellulose chain (4). In contrast, visualization of the secondary wall CSCs has remained difficult (7), largely because secondary walls are produced around cells that are situated deep in plant tissues and that are thus obscured from visualization by conventional confocal microscopy.

Instead of attempting to visualize the secondary wall CSCs in tissues where secondary wall synthesis normally occurs, Watanabe *et al.* made use of a system that allows secondary cell walls to be produced in all cell types, including the epidermal cell layers in *Arabidopsis* seedlings that are well suited for imaging. The secondary wall-inducing system is driven by a hormone-inducible transcription factor, VASCULAR NAC-DOMAIN7 (VND7), which can activate the complete cassette



Primary and secondary wall cellulose synthesis in plants. (A) Electron micrograph of an epidermal hypocotyl cell in an etiolated *Arabidopsis* seedling that produces primary wall cellulose. (B) Electron micrograph of a crude section through a full-grown *Zinnia* stem that displays the water-conducting vessel cells surrounded by thick secondary walls. Scale bars: 50 μ m (A) and 10 μ m (B).

of secondary wall-producing genes (8). By introducing a fluorescently labeled secondary wall cellulose synthase into this system, Watanabe *et al.* could clearly visualize secondary wall CSCs after hormone induction. The results show that the CSCs migrate with a speed of ~300 to 350 nm/min, significantly faster than the primary wall CSCs (4) (see the second figure, panel B). These findings raise

important questions regarding differences in the regulation and catalytic activity of primary and secondary wall CSCs.

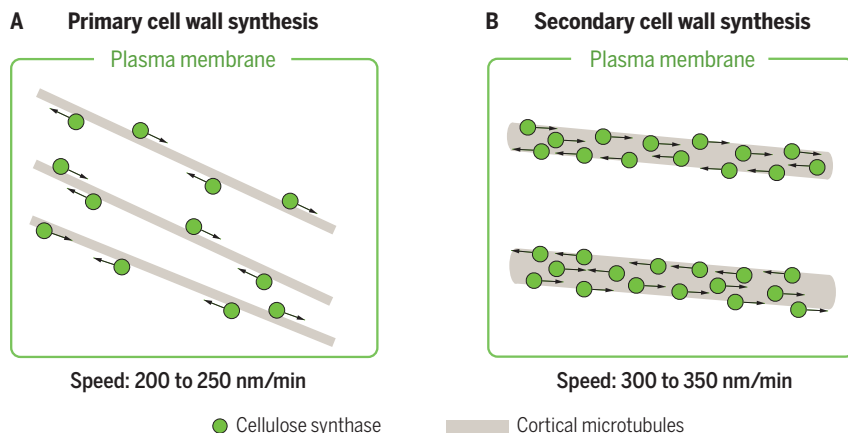
Secondary wall synthesis occurs in distinct spatial patterns that provide functional characteristics to the cells (9) (see the first figure, panel B). The VND7 transcription factor induces a spiraling secondary wall pattern (10), typically associated with the proto-xylem (i.e., the first-developing water-conducting xylem cells in a plant). Watanabe *et al.* show that the fluorescently labeled CSCs migrated along defined spiraling bands that were underpinned by thick bundles of the cytoskeletal component microtubules at the cell cortex (see the second figure, panel B). When the microtubules were disrupted with the microtubule-disrupting agent oryzalin, the CSCs were dispersed evenly in the plasma membrane; the speed of the CSCs and the amount of cellulose produced remained unchanged. The primary wall CSCs have been shown to track along cortical microtubules, and the microtubules are here believed to guide the direction of synthesis (3). However, the speed of the primary wall CSCs is not affected by removal of the microtubules. Hence, both the primary and secondary wall CSCs are directed by the cortical microtubules, suggesting that a related guiding principle underpins both processes.

Watanabe *et al.*'s visualization of secondary wall CSCs opens possibilities to explore regulatory processes that affect secondary wall cellulose production. It will be interesting to examine how such regulatory processes differ from, or align with, what is known from primary wall cellulose synthesis. For example, why do the primary wall CSCs move slower than the secondary ones, and what is the regulatory framework that underpins these differences? Furthermore, the VND7 secondary wall-inducing system provides a valuable tool to explore and appreciate the cell biology of secondary wall formation in plants. Introgression of secondary wall mutants into the VND7 line may reveal how the mutations affect the ability to produce secondary wall cellulose. Given that secondary wall cellulose contributes the bulk of a plant's biomass, the study of Watanabe *et al.* has removed a major brick in the wall toward our understanding of how plants produce cellulosic biomass. ■

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Why speed matters. Schematic view of primary (A) and secondary (B) wall cellulose synthases at the plasma membrane, where they are active. The cellulose synthases move through the membrane as a result of their catalytic activity, and the movement is typically guided by cortical microtubules. Watanabe *et al.* show that the secondary wall cellulose synthases move significantly faster than the ones forming the primary walls. The microtubule organization affects cellulose synthase distribution and causes cellulose to be produced evenly around cells during primary wall synthesis but in specific patterns (shown here as banded patterns) during secondary wall synthesis.

CANCER

The odds of immunotherapy success

Mutation load correlates with the response of melanomas to immunotherapy

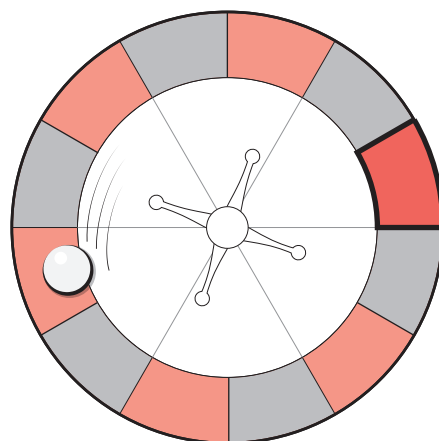
By **Matthew M. Gubin** and
Robert D. Schreiber

Cancer immunotherapy has advanced to the forefront of molecular medicine as a consequence of the success of monoclonal antibodies (mAbs) that block immune checkpoints. Such antibodies, like ipilimumab, reverse cancer-induced immunosuppression and induce durable therapeutic responses in certain cancer patients (1). However, because only some patients respond to checkpoint blockade therapy, there is a need for reliable biomarkers that identify individuals most likely to respond to such treatment. On page 207 of this issue, Van Allen *et al.* (2) report the genomic analyses of tumors from 110 melanoma patients prior to ipilimumab therapy. The study not only validates features of responsive melanomas suggested in smaller-scale analyses, but also refutes claims that associate responsiveness to ipilimumab with tumor antigens that show putative similarities to microbial proteins (3).

During “cancer immunoediting,” tumor cells may evolve antigens with a reduced ability to provoke an immune response and/or establish an immunosuppressive tumor microenvironment (4). Both characteristics are interrelated because the latter can arise as a consequence of chronic but ineffective T cell stimulation. These events trigger an inhibitory signaling pathway in T cells, involving the expression of immunosuppressive immune checkpoint proteins [such as cytotoxic T lymphocyte antigen-4 (CTLA-4) or programmed cell death-1 (PD-1)] that impair T cell effector mechanisms (1). Indeed, mAb blockade of CTLA-4 on T cells promotes their antitumor activity. Despite the success of checkpoint blockade therapy, many patients do not benefit from treatment for reasons that are unclear but may be related to the reduced antigenicity of tumor cells. Tumor antigens can be either aberrantly expressed normal proteins or abnormal proteins displaying tumor-specific expression (5). Although somatic cancer mutations could give rise to “nonself” tumor-specific mutant antigens (neoantigens), this concept had to await experimental demonstration of its generalization. Next-generation sequencing and

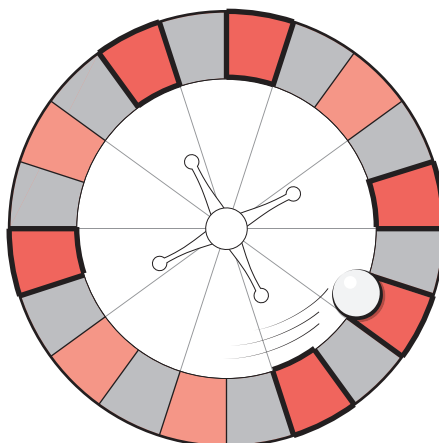
Nonresponders

Cancer patients with fewer mutations have a lower chance of responding to therapy



Responders

Cancer patients with more mutations have a higher chance of responding to therapy



 Mutations  Ipilimumab-responsive mutation

Neoantigen roulette. Melanomas with fewer mutations are less likely to contain “winning” neoantigen(s) and are thus more likely to be unresponsive to immunotherapy (ipilimumab). Those melanomas with greater numbers of mutations have an increased chance of responding to immunotherapy because they have an increased chance of having neoantigens that activate T cells. Functional activity of these T cells can be sustained by blocking CTLA-4 (with ipilimumab), thereby rendering the tumor susceptible to elimination by the immune system.

epitope prediction algorithms identified neoantigens in mouse tumors that functioned as tumor-specific targets for T cells (6, 7). Clinical studies showed that neoantigens were recognized by preexisting T cells in melanoma patients and were likely the major contributor to positive clinical effects seen with adoptive immune cell transfer therapy. Mouse models established that neoantigens were the targets of T cells activated by checkpoint blockade therapy, and that synthetic long peptides comprising these neoantigens were effective when administered as vaccines with CTLA-4 and/or PD-1 mAbs (8). Subsequent studies implied that cancers with higher mutation burdens, and therefore a greater likelihood of expressing neoantigens, were most likely to respond to checkpoint blockade therapy (5). Indeed, in melanoma, both the numbers of mutations and neoantigens correlated with patient response to ipilimumab (3). Further studies suggested a similar relationship among mutational burden, neoantigens, and response to PD-1 mAb therapy in non-small cell lung cancers (9) and colorectal cancers (10).

Van Allen *et al.* found that the number of nonsynonymous mutations per tumor correlated with clinical responses in ipilimumab-treated melanoma patients. They then pipelined their mutational data into algorithms to determine whether the number of potential neoantigens recognized by T cells correlated with clinical response more strongly [the algorithms pertained to binding of peptides to major histocompatibility complex class I (MHC-I), which are then presented to T cells]. Although there was a correlation, no increase in significance was observed; in fact, the mutational load itself was a better indicator of response ($P = 0.0076$ for mutational load versus $P = 0.027$ for neoantigens). An intriguing explanation for the latter observation surrounds the focus of Van Allen *et al.* on neoantigens formed by missense point mutations while not considering other potential alterations that could also lead to neoantigen formation [e.g., insertions/deletions, generation of fusion proteins, or posttranslational modifications]. Along similar lines, another study has suggested that any relationship between neoantigens and patient responsiveness to checkpoint blockade therapy take into account both MHC class I and class II neoepitopes (5).

Van Allen *et al.* further analyzed gene

Department of Pathology and Immunology, Washington University, St. Louis, MO, USA. E-mail: mgubin@path.wustl.edu

expression in a subset of tumors and demonstrated that an increased transcript expression of PD ligand 2 (PD-L2) and an immune “cytolytic” gene signature correlated with neoantigen load and tumor response to ipilimumab. Interestingly, the expression of CTLA-4 was an indicator of response, with both CTLA-4 and PD-L2 likely expressed in the tumor-infiltrating immune cells. These findings may reflect ongoing preexisting T cell responses, as an inflamed tumor micro-environment prior to ipilimumab treatment has been associated with response (11). Thus, even though the numbers of mutations and neoantigens correlate with response, it may be important to consider biomarkers that reflect a tumor’s immune context (12). Indeed, the expression of PD-L1 on tumor-infiltrating immune cells has been put forward as a possible biomarker in PD-L1 mAb treatment (13). Other factors such as increased density and decreased diversity in antigen specificity of T cells within the tumor may also provide predictive value, as observed in melanoma patients treated with PD-1 mAb (14).

Van Allen *et al.* also investigated whether any recurring neoantigens or neoantigens with shared features might better predict ipilimumab response. Nearly all neoantigens were patient-specific and most likely represent “passenger” mutations that do not directly contribute to tumorigenesis. Examination of neoantigens did not reveal any features or motifs exclusive to responders.

Notably, the results of Van Allen *et al.* do not support the notion of a four-amino acid (tetrapeptide) motif within the predicted neopeptides of melanoma patients who received durable clinical benefit from ipilimumab (3). Van Allen *et al.* combed for this motif within their data set and found no enrichment of this signature in the clinical benefit cohort. Questions have been raised (15) about the validity of this tetrapeptide signature, as it violates widely accepted rules governing antigen presentation and T cell recognition. Further examination should clarify whether the concept is indeed correct or dangerously misdirecting the field. ■

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HISTORY OF SCIENCE

Beyond the “Mendel-Fisher controversy”

Worries about fraudulent data should give way to broader critiques of Mendel’s legacy

By Gregory Radick

One hundred and fifty years ago, Gregor Mendel delivered his lectures on “Experiments on Plant Hybrids,” going on to publish them in 1866 (1). Around the world, celebrations of the monk whose work with pea varieties made him the father of genetics are under way. Mendel has alas acquired another, less auspicious title, as “the father of scientific misconduct,” owing to suspicions that he faked some of his data (2). The suspicions have turned out to be groundless (3, 4). Along the way, however, they not only

“Mendel’s program of experiments thus became, in Fisher’s words, ‘a carefully planned demonstration of his conclusions.’”

damaged Mendel’s reputation unfairly but, as a look at the history of the controversy shows, sent critical discussion of his data down a sidetrack.

The Mendel-Fisher controversy, as it is known, takes its name from a 1936 paper by the Cambridge statistician and theoretical geneticist Ronald Fisher (5). But the discovery that Mendel’s data conform improbably closely to the predictions of his theory—that they are “too good to be true”—was due not to Fisher but to a scientist from the previous generation, the Oxford biologist W. F. R. Weldon. “About pleasanter things, I have heard of and read a paper by one Mendel on the results of crossing peas, which I think you would like to read,” Weldon wrote to the mathematician Karl Pearson in October 1900, only a few months after Mendel’s paper had been rediscovered (6). Over the next year, Weldon grew skeptical. The more he learned about pea varieties and their pedigrees, the more convinced

he became that Mendel’s “laws” had no validity beyond the artificially purified races Mendel worked with, and that the binary categories that Mendel used to classify pea characters—green or yellow for seed color, round or wrinkled for seed shape, and so on—obscured a far more variable reality.

While preparing a paper setting out his concerns, Weldon checked the “probable error” of Mendel’s results, using a standard formula to calculate expected deviations from the theoretically predicted values given the number of observations made. For example, Mendel had reported that in the offspring of the hybrid pea plants, 5474 out of 7324 seeds had the dominant character of roundedness—a figure extremely near to the predicted 75% for a sample of that size. Most of Mendel’s other data sets showed similarly close agreement with his theory. “He is either a...liar, or a wonderful man,” judged Weldon in a letter to Pearson in November 1901 (7). In his published paper, which also made use of Pearson’s new chi-squared test, Weldon stressed the improbable nature of Mendel’s results. Run Mendel’s experiments again at the same scale, Weldon reckoned, and the chance of getting worse results is 16 to 1 (8).

For Weldon, the data problem was of interest as a symptom of a much deeper problem: the binary categories Mendel had used, and the oversimplified theory of dominance he had erected on their basis. In the book-length manuscript where Weldon discussed the 1866 paper most fully, he did not even mention his previous analysis of probable error (9). What he dwelt on, at length, was the mounting evidence against anything like a Mendelian view of dominance as something an inherited character possesses independently of its developmental context. The effect of the same bit of chromosome on a body can be different depending on the hereditary background and the wider environmental conditions. The manifest character can be dominant, or recessive, or neither.

Weldon was at work on the book manuscript in 1904 to 1905, while in full battle mode with Pearson and others against the growing corps of “Mendelians” led by William Bateson. At Weldon’s death in 1906, the

School of Philosophy, Religion and History of Science,
University of Leeds, Leeds, UK. E-mail: g.m.radick@leeds.ac.uk

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A plate of peas. In this photographic plate from Weldon's 1902 article (8), images 1 to 6 and 7 to 12 show a color scale from green to yellow in the seeds of two hybrid pea varieties (with the seed coats removed). Images 13 to 18 show color variation in cotyledons of the same seed, and 19 to 24 show differences between the color of a seed's coat and its cotyledons (though Weldon was not happy with the colors as published). Another, black-and-white plate displayed degrees in the development of wrinkles. Weldon's point was that inherited characters are diverse in ways that a Mendelian perspective, indifferent to developmental context, neither acknowledges nor accounts for.

manuscript was still unfinished and unpublished. It is thus no wonder that his larger critique was ignored and the importance of context, and the variability it brings, generally paid no more than lip service (10). [Bateson late in life cheerfully admitted that “scientific Calvinism” struck him as a fair summary of Mendelism's message (11).]

Even so, the more statistically minded Mendelians took heed of Weldon's data analysis (12). One was the young Fisher, who, in a talk on heredity in 1911, spoke about the 16-to-1 odds that Weldon first calculated (13). When asked in the mid-1930s to contribute to a new journal in the history of science, Fisher made the problem his own. However, he drew a very different lesson from the Mendel case than Weldon had.

Reanalyzing Mendel's data statistically, Fisher, too, found that they are improbably good. But what that showed, Fisher argued, was what a great thinker Mendel was. Relatively soon after the crossing experiments were begun, Mendel must have worked out his theory in the abstract. From that moment, Mendel knew how his data ought to look. Mendel's program of experiments thus became, in Fisher's words, “a carefully planned demonstration of his conclusions.” For Fisher, the data's shortcomings were thus largely to Mendel's credit. Such blame as Fisher was willing to consider he meted

out to a well-meaning but misguided underling, who, Fisher surmised, must have quietly got rid of whatever plants threatened to mess up the master's ratios: “Mendel was deceived by some assistant who knew too well what was expected” (5).

Although, like Weldon, Fisher expressed himself more pungently in private correspondence, his paper was intended to settle rather than spark controversy. There was no “Mendel-Fisher controversy” for decades, even as Fisher succeeded in raising the profile of the need for statistical evaluations of goodness of fit in genetics and other areas of research. Only toward the end of the 1960s did Fisher come to be understood as having leveled an accusation of fraud. Quite why that happened, and why the accusation then became so widely known, are matters for ongoing historical inquiry. What is plain is that Fisher's analysis had a far greater prominence in the publications near the centenary of Mendel's paper (1965 to 1966) than in those around the 1950 Golden Jubilee of genetics (14). In 1950, genetics was under immense political pressure due to the influence of Mendelism-rejecting Trofim Lysenko in the Soviet Union. Unsurprisingly, Western geneticists chose not to emphasize concerns about Mendel's data. Only from the mid-1960s, Lysenkoist biology was in terminal

decline, did those concerns begin to be aired.

But now the Cold War is long gone, and the consensus view after half a century of debate is more or less what it was at the start: Mendel's data are indeed improbably good, but that in itself is not evidence of fraud, nor is there any other evidence to suggest fraud (3). So should we let the matter drop? That would be a missed opportunity. Undoubtedly Mendel suffered from unconscious bias, counting as yellow what ought to have counted as green when it supported his theory (4). But stopping there would leave untouched the question of whether Mendel was right to work with just the two categories in the first place, and the connections between those categories and the absence of the developmental context from the traditional Mendelian picture—a picture that remains central to education in genetics. It has proved very hard to “unthink” determinist Mendelism, even as genetics in the 21st

century goes ever further in disclosing the importance of variability, interaction, complexity, and even ancestry (15). If the time is ripe for retiring the problem of Mendel's data, it is also ripe for rediscovering, and engaging with, Weldon's critique of Mendelian concepts. ■

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From transient infection to chronic disease

Can some infections “scar” the immune system?

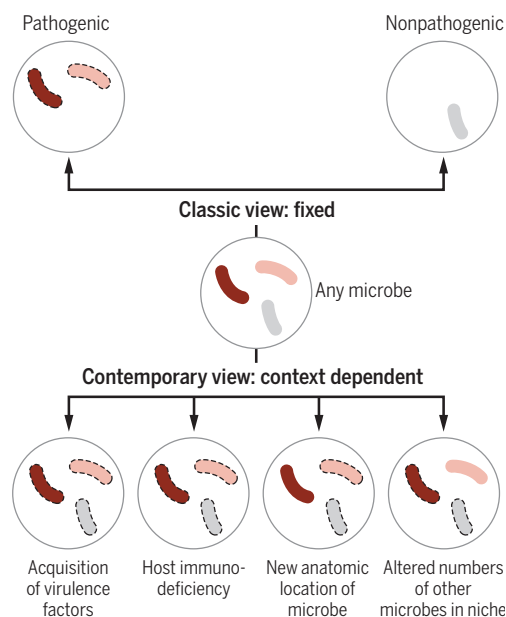
By Carl Nathan

An infection is considered to be resolved when the causative microbe has been cleared and host structure and function return to normal—except, perhaps, for a scar in the affected tissue. In a recent study, da Fonseca *et al.* (1) argue that some resolved infections leave scars of another sort—long-lasting immune dysfunction. The authors propose that “immunologic scarring” by resolved infections may account for a wide range of chronic diseases.

Paving the way to this concept are chronic diseases whose origin had been mysterious for decades, until an ongoing bacterial infection was identified as causal, such as gastric and duodenal ulcers caused by *Helicobacter pylori*. However, inflammatory bowel disease, rheumatoid arthritis, sarcoidosis, and other inflammatory or autoimmune diseases remain unexplained despite searches for a specific infectious cause (2). Da Fonseca *et al.* propose that a long-gone infection may set the stage for chronic disease by impairing the immune system's checks and balances. They base this proposal on the observation that most of the mice that had cleared a gastrointestinal tract infection by *Yersinia pseudotuberculosis* within 3 weeks had persistently leaky lymphatic vessels draining the intestine and persistently enlarged, inflamed lymph glands in the mesentery (a broad membrane in the abdomen). These changes lasted up to 42 weeks (the last period examined), which is roughly equivalent to 28 years of a human life span. Specific populations of immune response-guiding cells called dendritic cells were missing from the mesenteric lymph glands and appeared instead in the mesenteric adipose tissue. The adipose tissue accumulated other immune cells as well, and produced inflammatory cytokines such as tumor necrosis factor and interleukin-1, much like visceral adipose tissue in obese individuals. In obesity, inflammation in visceral adipose tissue has deleterious effects on distant tissues (3), and a similar effect may have contributed to the immune dysfunction observed by da Fonseca *et al.*: The mice that re-

covered from *Yersinia* infection developed an immune response to a new protein in their food instead of becoming tolerant to it, and they responded feebly to vaccination. Thus, the complex regulatory balance between the immune system and the gastrointestinal microbiota that sustains health was apparently converted by a brief, resolved infection into a state of chronic disease marked by altered structure and function of mesenteric lymph vessels, lymph glands, and adipose tissue.

These observations add to earlier evidence



Bacterial pathogenicity. In the classic view, bacteria were classified as pathogenic or nonpathogenic, according to their inherent properties. In a contemporary view, bacteria that are usually nonpathogenic can become pathogenic depending on their biological context.

of long-standing immunologic dysfunction and chronic disease following a resolved infection. Infected mice cleared a viral infection, but went on to develop an asthma-like disease (4) mediated by sustained activity of natural killer T cells driving macrophages to produce interleukin-13 (5).

Although the concept proposed by da Fonseca *et al.* is established, the post-*Yersinia* disorder they describe may actually illustrate a different phenomenon: context-dependent pathogenicity. Medical microbiologists used

to consider pathogenicity as an inherent property of some but not other bacterial species (see the figure). However, it is now recognized that some bacterial species that are usually nonpathogenic may cause disease when the host becomes immune-deficient or when the bacteria occupy a new location in the host, acquire virulence factors through infection by a plasmid, and/or face a sudden lack of competition from their usual microbial neighbors.

What raises the issue of context-dependent pathogenicity in the study of da Fonseca *et al.* is their observation of persistent, suppurative infection of the mesenteric lymph glands by bacteria other than *Yersinia*, chiefly lactobacilli. Lactobacilli are usually thought of as beneficial—people swallow them as probiotics. Yet the long-lasting immune dysfunction observed by da Fonseca *et al.* came to a halt when mice were treated with antibiotics, and was prevented by using germ-free mice for the *Yersinia* infection. The authors did not attribute the immune dysfunction to the chronic lactobacillus infection because they could reproduce some of the immune dysfunction in antibiotic-treated mice by feeding them nonviable microbial products. However, in mice not treated with antibiotics, the invasive lactobacilli could well have been the functionally relevant source of microbial products that sustained the immune dysfunction. The failure to eliminate the lactobacilli from the lymph glands despite extensive infiltration by neutrophils—the body's most powerful bacteria-killing cells—suggests that the lactobacilli may have acquired some of the immune-evading, -suppressing or -resisting hallmarks of pathogens. Thus, the persistent immune dysfunction observed by da Fonseca *et al.* may indicate that acute infection by a known pathogen can create a context for an otherwise innocuous microbe to become a chronic pathogen.

Whatever the explanation, the observations of da Fonseca *et al.* are likely to breathe new life into the search for infectious provocateurs of chronic immune and inflammatory disorders, whether the trigger is a transient infection by a known pathogen or a cryptic, chronic infection by a microbe that is usually nonpathogenic. ■

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Weill Cornell Medical College, New York, NY 10065, USA.
E-mail: cnathan@med.cornell.edu

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MEDICINE

Membrane traffic en route to cancer

The spatial organization of internal membranes influences receptor signaling and disease

By Shawn M. Ferguson

Binding of growth factors to cell surface receptors initiates complex intracellular signaling cascades that promote cell growth, survival, and proliferation (1). The membrane composition and spatial arrangement of intracellular organelles represents a critical network that influences the transduction of such signals. This close relationship blurs the boundaries of classifying proteins into simple categories such as signaling and membrane trafficking. The challenge of maintaining such strict distinctions is illustrated on page 211 of this issue by Wheeler *et al.* (2), who identify oncogenic functions for Rab35, a protein involved in regulating endosomal membrane traffic (3).

The excessive activity of growth factor receptor signaling pathways is a patho-

membranes to help concentrate and organize signaling modules in specific subcellular locations.

Wheeler *et al.* identified the new function for Rab35 in a genetic screen for novel regulators of Akt [also known as protein kinase B (PKB)], a protein kinase that integrates multiple signals in the class 1 phosphatidylinositol 3-kinase (PI3K) signaling pathway (1). Growth factor receptor stimulation recruits PI3K from the cytosol to the plasma membrane (either through direct interaction with the receptor or via protein adaptors). Here, it can phosphorylate the 3 position of the inositol ring of phosphatidylinositol 4,5-bisphosphate [$\text{PI}(4,5)\text{P}_2$] (a lipid that is predominantly found in the plasma membrane) to yield $\text{PI}(3,4,5)\text{P}_3$. Multiple signaling proteins contain pleckstrin homology (PH) domains with selective affinity for binding to $\text{PI}(3,4,5)\text{P}_3$ or $\text{PI}(3,4)\text{P}_2$ [produced by the action of 5-phosphatases on $\text{PI}(3,4,5)\text{P}_3$] and are thus recruited to the plasma membrane in response to synthesis of this lipid.

Membrane recruitment via PH domain- $\text{PI}(3,4,5)\text{P}_3$ interactions promotes downstream signaling by controlling both the local concentration and activity of signaling proteins, such as phosphoinositide-dependent protein kinase 1 (PDK1), Akt, and mechanistic target of rapamycin complex 2 [mTORC2 (Sin1 subunit)] (5, 6). Upon their plasma membrane recruitment and

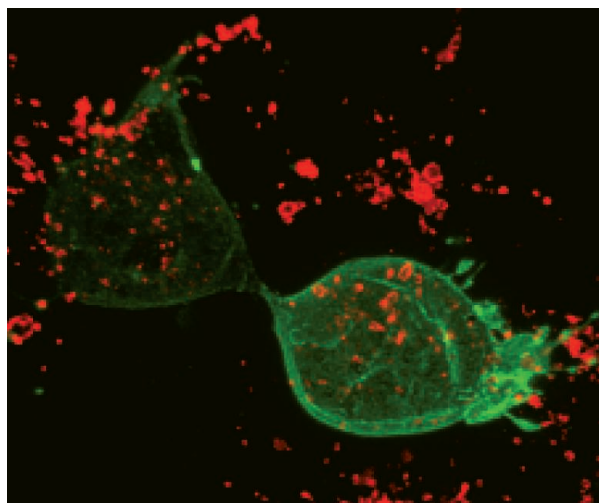
activation, PDK1 and mTORC2 both phosphorylate distinct sites on Akt that modulate its ability to engage diverse downstream targets that promote cell growth, proliferation, and survival (1).

Wheeler *et al.* took advantage of this synergistic activation of Akt by PI3K-dependent signals to perform a lentiviral short hairpin RNA screen for genes that regulate PI3K activity. This strategy identified Rab35 as important for supporting normal growth factor-induced Akt phosphorylation and activation. The existence of predicted activating Rab35 mutations in cancer sequence

databases provided a further rationale for investigating the contributions of Rab35 to the PI3K-Akt signaling pathway.

The Rab family of small guanosine triphosphatases (GTPases) comprises more than 60 different members that are best characterized for their roles in regulating numerous distinct intracellular membrane trafficking reactions (7). Rab35 has been specifically implicated in physiological processes that depend on recycling of proteins from early endosomes (3). Spatial control of Rab35 function is conferred by the Conectendin/DENND1 family of guanine nucleotide exchange factors (GEFs) that promote the loading of Rab35 with GTP (3, 8). Through interactions with clathrin and its adaptor, AP-2, at plasma membrane clathrin-coated pits, these GEFs ensure the presence of activated Rab35 on early endosomes (3) (see the figure). At these endosomes, Rab35 interacts with specific effectors involved in the actin cytoskeleton, lipid metabolism, and protein trafficking to ensure the recycling of multiple receptors and cell adhesion proteins back to the plasma membrane, so as to limit their delivery to and degradation by lysosomes (3). Although several GTPase-activating proteins (GAPs) have been identified that limit Rab35 function (3), the underlying mechanisms that direct their spatial and temporal specificity are less well understood.

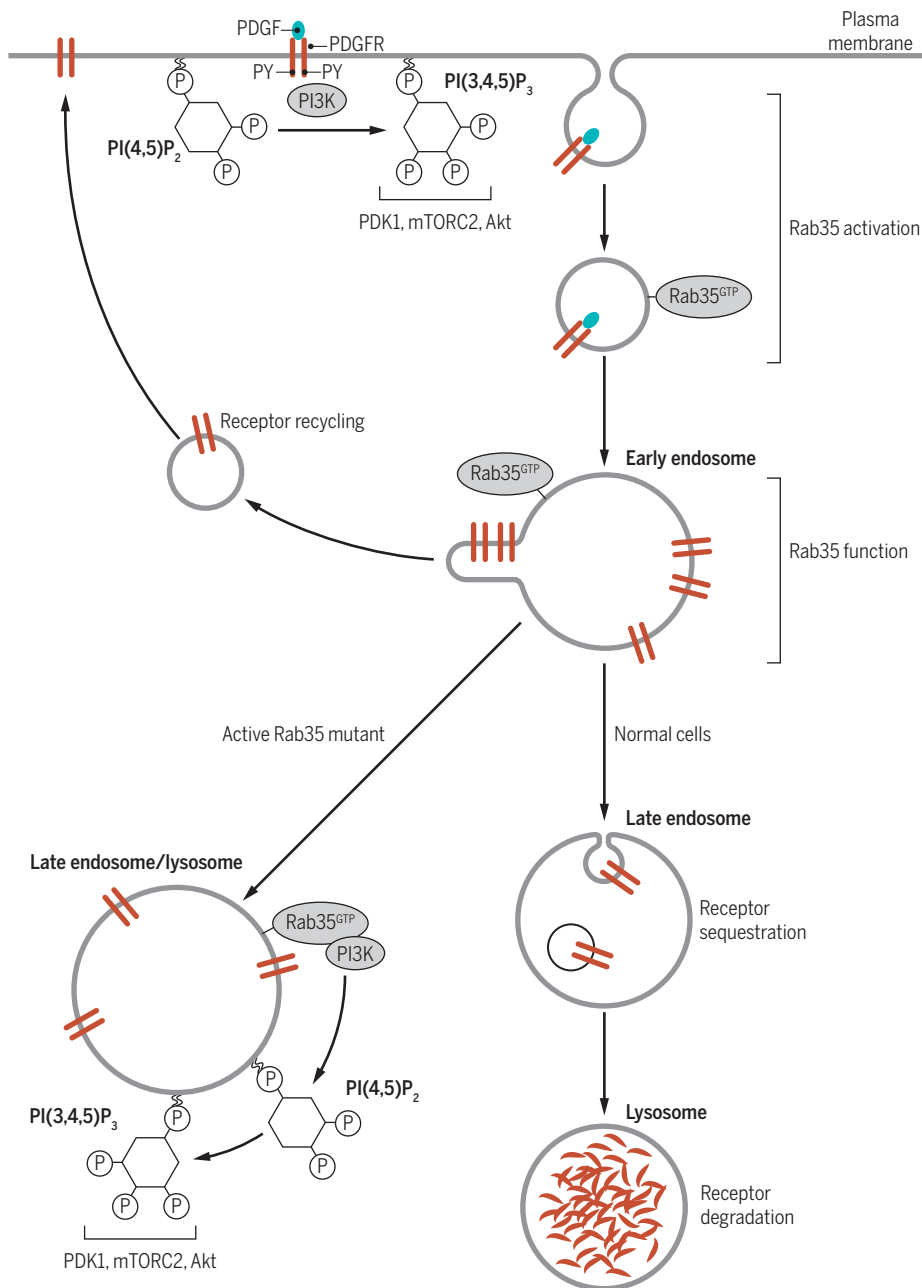
Wheeler *et al.* demonstrated that loss of Rab35 impairs growth factor-stimulated activation of the PI3K-Akt signaling pathway. Conversely, expression of Rab35 mutants that favor the active, GTP-bound, state (including those identified in human cancers) promoted PI3K activity and protected cells against growth factor deprivation by maintaining a high basal level of Akt activation. Through an elegant series of experiments, the actions of constitutively active Rab35 were placed upstream of PI3K in the signaling pathway. In fact, the GTP-bound form of Rab35 copurified with PI3K, suggesting a function that is spatially close to PI3K. This observation brings to mind the critical role for the Ras GTPase in supporting PI3K signaling in development and cancer (9). Rab5 has also been shown to interact with PI3K and stimulate its activity (10), thus raising mechanistic questions about how the actions of these different GTPases toward PI3K relate to one another.



Membrane bound. Growth factor receptors like PDGFR α (green) localize to the cell membrane in the absence of their ligand.

genic mechanism in many forms of cancer (4). This has motivated major efforts to understand the mechanisms that propagate such signals within cells and how modulating these pathways might yield therapeutic benefits. It is increasingly appreciated that intracellular signaling pathways rarely rely on random, diffusion-mediated encounters between signal transduction proteins but instead depend heavily on intracellular

Department of Cell Biology and Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University, New Haven, CT 06510, USA. E-mail: shawn.ferguson@yale.edu



Membrane traffic control. Activated receptor tyrosine kinase growth factor receptors (including PDGFR) bind ligand and recruit PI3K to the plasma membrane to trigger the conversion of PI(4,5)P₂ to PI(3,4,5)P₃, which recruits and activates PDK1, mTORC2, and Akt (**top middle**): PY, phospho-tyrosine. Activated receptors are also subject to internalization by both clathrin-dependent and independent pathways (**top right**). The clathrin-mediated endocytosis machinery also activates Rab35, an important regulator of recycling from early endosomes. Receptors that are not recycled (**top left**) at early endosomes are subject to sequestration in the intraluminal vesicles of late endosomes and subsequently degraded in lysosomes (**bottom right**). The study by Wheeler *et al.* suggests an alternative role for late endosomes or lysosomes as sites of PDGFR signaling in cells that express active Rab35 mutants (**bottom left**).

Of multiple growth factor receptors examined, the platelet-derived growth factor receptor (PDGFR) pathway was found to be particularly sensitive to Rab35 activity, and PDGFR itself was constitutively activated in cells expressing active Rab35 mutants. In contrast to the canonical role for PDGFR-PI3K signaling at the plasma membrane

and Rab35 actions early in the endocytic pathway, active Rab35 mutants and the PDGFR were surprisingly both enriched at lysosomes when coexpressed, regardless of whether cells had been stimulated with PDGF (see the figure). Meanwhile, in the absence of constitutively active Rab35, PDGFR accumulated at lysosomes only af-

ter acute PDGF treatment. Thus, the localization of PDGFR to lysosomes in Rab35 mutant cells is consistent with Rab35's persistently activated state.

Lysosomal localization of growth factor receptors after stimulation is normally linked to their sorting into intraluminal vesicles and subsequent degradation. The lysosome thus seems like an unlikely site for PDGFR to signal via the PI3K pathway. However, despite the old-fashioned view that lysosomes are simply the "garbage can" of the cell, they are now increasingly appreciated as an important signaling platform for the mTORC1 pathway (11, 12). Even though such endosomal membranes have not traditionally been viewed as sites that contain appreciable levels of PI(4,5)P₂ (the preferred substrate of PI3K), recent studies support the existence of a pool of PI(4,5)P₂ on endosomal membranes that regulates growth factor receptor sorting and degradation (13). In this light, a novel role for the lysosome in PDGFR-PI3K signaling is an intriguing possibility that warrants further investigation.

This new study raises questions about the roles played by Rab proteins in cancer. In parallel, another recent study revealed a critical role for Rab13 in the invasive behavior of epithelial cells, with implications for cancer metastasis (14). Collectively, these advances point to the importance of better understanding the relationships between endocytic membrane traffic, the PI3K signaling pathway, and the aberrant properties of cancer cells. Such studies also raise questions about how the membrane architecture that is sculpted by membrane trafficking reactions may have a broader impact on other signaling pathways in health and disease. ■

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CATALYSIS

The importance of being together

Controlling the coordination of platinum boosts catalytic reaction rates

By Ifan E. L. Stephens,^{1,2} Joseph S. Elias,¹ and Yang Shao-Horn^{*1}

Platinum catalyzes several chemical and electrochemical reactions that are central to humankind's use of energy, such as the electrochemical reduction of molecular oxygen, carbon monoxide oxidation, and the water-gas shift reaction to produce carbon dioxide and hydrogen from carbon monoxide and water. Two reports in the current issue provide insights into which platinum coordination number—defined as the number of nearest neighbors—may provide the highest catalytic activities for these reactions. On page 185, Calle-Vallejo *et al.* use the concept of generalized coordination numbers to show that concave atomic sites on a platinum sin-

gle crystal are highly active for oxygen electroreduction (1). On page 189, Ding *et al.* use spectroscopic techniques to determine what role isolated Pt atoms play in CO oxidation and the water-gas shift reaction (2).

Generally speaking, the lower the coordination number, the stronger the surface interacts with reaction intermediates (3). Maximum reaction rates can be obtained when the binding of reaction intermediates to the surface is neither too strong nor too weak, yielding a "Sabatier volcano" (see the figure) named after the French chemist Paul Sabatier. Lowering the coordination number of platinum by increasing the number of steps on single-crystal platinum surfaces leads to a slight increase in the rates of electrochemical oxygen reduction (4, 5). On the other hand, increasing the number of steps on platinum surfaces can boost the rates of CO oxidation substantially (6–8). This observation has motivated the preparation of CO oxidation catalysts that minimize the coordination number of platinum through

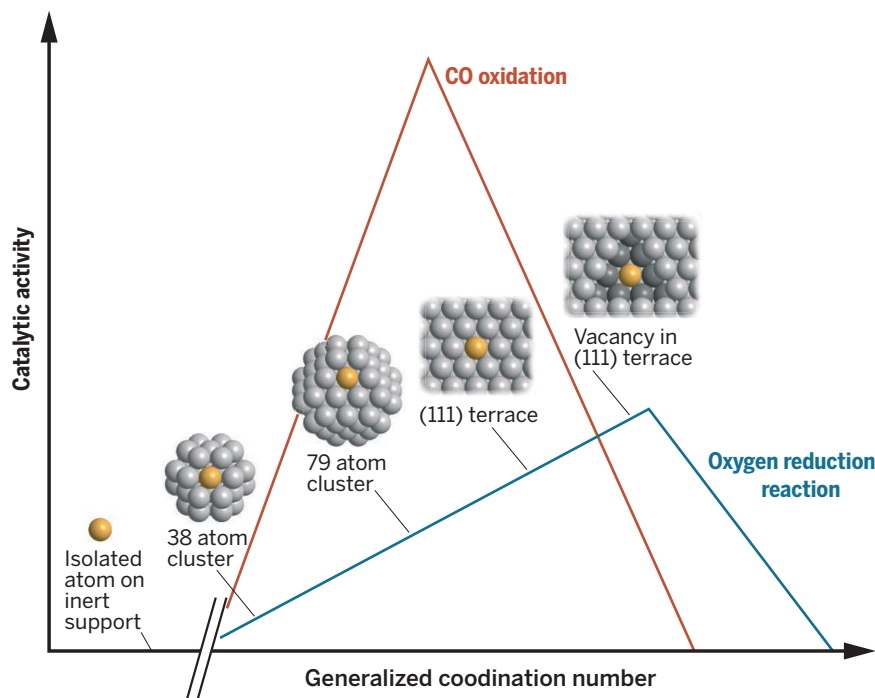
use of small clusters and isolated atoms on oxide supports (9).

Calle-Vallejo *et al.* now predict that concave surface sites should exhibit higher catalytic activity than a Pt(111) surface due to their reduced binding strength with reaction intermediates. Such concave sites may be obtained by placing Pt atoms in a cavity with increased coordination relative to a Pt(111) surface. The study builds on previous work reporting that the binding between a Pt(111) surface and OH, the key reaction intermediate, is slightly too strong for optimized oxygen reduction (10). Accordingly, increasing the coordination number should increase the catalytic activity. Similarly, ligand (11) and strain effects (12) from alloying with platinum also weaken the binding of OH, thus increasing catalytic activity (13).

Calle-Vallejo *et al.* use the concept of generalized coordination number ($\overline{CN}$), which takes into account interactions with next-nearest neighbors as well as nearest neighbors. Because $\overline{CN}$ linearly correlates with binding energies of reaction intermediates, it can be used to describe catalytic activity (see the figure). The authors performed simple, yet elegant, electrochemical treatments to engineer such defects into a Pt(111) surface. The oxygen reduction activity of these surfaces was three times that of bulk Pt(111). Such metastable concave structures may be responsible for the high activities of nanocatalysts such as dealloyed nanoparticles, hollow nanoparticles, and Pt-Ni nanoframes (14).

Generalized coordination numbers are simple and intuitive. Moreover, in combination with prior knowledge of the relationship between binding energies and reaction rates for any given reaction (10), they clearly have predictive power. Further work is needed to provide direct evidence for the concave structures—for instance, using scanning tunneling microscopy. Moreover, the $\overline{CN}$ descriptor could be developed to incorporate the effect of water coadsorbed with OH; this would likely account for the activity of stepped Pt surfaces for oxygen reduction (4, 5).

Ding *et al.* explore the nature of the active sites on oxide-supported platinum catalysts that catalyze both CO oxidation and the water-gas shift reaction. Using elegant site-specific techniques that probe the entirety of the catalyst, they show that



A coordinated effort. Platinum sites with higher coordination numbers (highlighted in gold) turn over oxygen reduction catalysis faster than those with lower coordination numbers. Generalizing this concept to include the coordination of the nearest-neighbor platinum sites, Calle-Vallejo *et al.* were able to predict that concave sites are three times as active for oxygen electroreduction as is Pt(111). This strategy may be extended to CO oxidation (red) and the water-gas shift reactions, investigated by Ding *et al.*, who show that platinum nanoparticles rather than site-isolated platinum atoms are responsible for faster rates.

isolated platinum atoms on oxide supports bind CO too strongly to be active. Rather, the authors find that metallic platinum sites on nanoparticles catalyze the reactions. Framing these results in terms of the coordination number, single platinum atoms correspond to the strong-binding regime at the left of the figure. The more highly coordinated sites on small nanoparticles are closer to the apex of the figure.

In contrast to previous work (9), Ding *et al.*'s results suggest small metallic Pt nanoparticles, rather than isolated Pt atoms, give rise to enhanced catalysis of CO. The applicability of these findings will depend on the elemental composition of the catalyst and electronic interactions between the support and the metal. For example, unlike platinum, making gold catalytically active requires atomically dispersed atoms rather than larger nanoparticles (15). Further work is needed to identify which exact sites on platinum nanoparticles (for example, terrace, edge, kink, or corner sites) are active for CO oxidation and water-gas shift catalysis. Of particular interest is the exploration of relationships between the rates of the CO oxidation/water-gas shift reactions on platinum and the generalized coordination number introduced by Calle-Vallejo and co-workers (see the figure).

The implications of reaction rate sensitivity to coordination number for heterogeneous catalysis are both subtle and far-reaching. The rational design of catalysts for heterogeneous processes requires a detailed understanding of the interplay of both the electronic structure of the catalyst surface and its local coordination environment. The insights described in (1, 2) highlight the immense opportunities for catalyst discovery and improvement provided by detailed understanding of the nature of active sites. ■

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FOOD SCIENCE

Designing a sustainable diet

Sustainability as dietary guidance created political debate

By Kathleen Merrigan,^{1*} Timothy Griffin,² Parke Wilde,² Kimberly Robien,³ Jeanne Goldberg,² William Dietz³

In the United States, a vigorous debate is under way over government-issued dietary guidance. A February 2015 report by the U.S. Dietary Guidelines Advisory Committee (DGAC) recommended, for the first time, that food system sustainability be an integral part of dietary guidance in the 2015 Dietary Guidelines for Americans (DGAs) (1). With the final decision from the secretaries of Health and Human Services (HHS) and of Agriculture (USDA) about what parts of the DGAC recommendations to include in the 2015 DGAs expected at the end of this year, we discuss the need to incorporate sustainability into dietary guidelines and the political maneuvering under way to excise it.

DGAs, which are updated every 5 years, have consistently recommended a diet higher in plant-based foods and lower in animal-based foods. This year DGAC concluded that “consistent evidence” suggests that such a dietary pattern is not only more healthful but also is associated with less environmental impact than the average American diet (1). This rationale has ignited controversy (2).

Dietary guidelines are not unique to the United States. The United Nations Food and Agriculture Organization (FAO) posts dietary guidelines by 67 national governments. The purpose of such guidance historically has been to educate people on how to avoid malnutrition. In the United States, DGAs are important information for nutrition professionals. Skeptics argue that DGAs are largely inconsequential. Adherence is problematic; only 4% of Americans meet DGAs, and

fewer than 40% of American adults meet the healthy weight recommendation (1, 3).

Nevertheless, DGAs have tangible influence on federal programs. DGAs inform meal content for example, for (i) military personnel; (ii) 8.6 million needy Americans served by the Women, Infants, and Children program; and (iii) 31 million children served through the National School Lunch Program. DGAC recommended that the government do a better job aligning fed-



eral nutrition assistance programs [e.g., the Supplemental Nutrition Assistance Program (SNAP)] with DGAs. There are no restrictions on how SNAP benefits can be used, although whether to limit benefits to healthy food purchases has been debated for years. If DGAC's recommendation is upheld, it would affect 47 million SNAP recipients and billions of dollars annually in government spending. The SNAP debate surely contributes to the DGA controversy.

SUSTAINABILITY. FAO defines sustainable diets as those with “low environmental impacts which contribute to food and nutrition security and healthy life for present and future generations” (4). By this or any other definition of sustainability, no

¹Trachtenberg School of Public Policy and Public Administration, the George Washington University, Washington, DC 20052, USA. ²Friedman School of Nutrition Science and Policy, Tufts University, Medford, MA 02155, USA. ³Milken Institute School of Public Health, the George Washington University, Washington, DC 20052, USA. *Corresponding author. E-mail: kmerrigan@gwu.edu

country has achieved a sustainable diet. Current and emerging dietary patterns threaten human health in developing and developed countries (5, 6) and negatively affect long-term food security (7). It is thus not unreasonable that government-issued dietary guidelines take sustainability into account. The Netherlands, Brazil, and Sweden have already done so. Germany and the United States have active, but unresolved, discussions.

As required by law, the DGAC based its report on scientific evaluations (4, 8). Opponents of the sustainability language assert that DGAC has overstepped its statutory bounds (2). But nothing in the 1990 DGA statute prevents inclusion of sustainability, and the DGAC argument that future food insecurity is predictable without attention to sustainability is relevant and compelling. Pending House and Senate appropriations bills that govern HHS and USDA propose new statutory language that would require the secretaries to consider diet and nutrient intake only, which would prevent sustainability considerations. DGAC sent a letter to

“The U.S. debate has awakened civil society... and has aligned public health and sustainability advocates.”

congressional appropriators protesting this “unduly narrow” restriction, which fails to consider topics already within the scope of DGAs, e.g., guidance on physical activity. It is increasingly likely that Congress will fail to pass these appropriations bills and instead will fund the government through a continuing resolution. Even in that scenario, the congressional language poses a serious challenge to the secretaries of HHS and USDA.

POLITICAL MANUEVERING. We believe the issue of scope is not the overarching concern but a political maneuver to excise sustainability from dietary discussions for four reasons. First, many industry leaders do not want any food disparaged, and a DGA process that evaluates sustainability will likely lead to conclusions that some foods are better than others. Although DGAs are advisory (not mandatory, except as previously described for government programs), the worry is that sustainability evaluations may lead to future regulation. Fear of regulation underlies industry protest of the current U.S. Food and Drug

Administration proposal to require labels for added sugar; industry may see transparency as a step toward a ban, as happened with trans fats.

The meat industry feels especially under attack. Much discussion of sustainable diets has focused on the increase in livestock production that will result from population growth and adoption of Western-style diets by an expanding middle class in the developing world. Whether from a health perspective (e.g., reducing coronary heart disease) or an environmental perspective (e.g., reducing methane emissions and deforestation) the dietary advice is the same: eat less meat (7). But reducing discussion to a meat-focused debate ignores larger points around food production. For example, it takes up to 2.8 liters of water to produce a single “heart-healthy” almond (9). With 80% of the world’s almonds grown in drought-stricken California, should consumers be advised to limit almond consumption and consider alternatives that consume fewer resources?

Second, sustainability has potential to move dietary guidance from a system based on food groups (e.g., fruits, vegetables, and protein) to individual foods within a food group (e.g., chicken versus beef). The environmental footprint may elevate certain foods over others. The Dutch 2011 dietary guidance presented four sustainability-related recommendations, including advice to eat two portions of fish per week (10). However, fishing has sustainability issues, and the recommendation was deemed “ecologically detrimental.” The Dutch Health Council is now evaluating the sustainability of individual fish species, with a new version of the dietary guidance expected this October.

Third, the sustainability discussion has potential to forge new political coalitions. The 2014 Brazilian dietary guidelines were adopted despite food industry protests over the recommendation to avoid ultra-processed food (e.g., chicken nuggets rather than freshly prepared chicken) (11). This bold approach may be attributed to engagement by civil society and the breakdown of the traditional coalition of farmers and agribusiness over ultra-processed food guidance, which garnered farmer support. The U.S. debate has awakened civil society to the potential influence of DGAs beyond food consumption and has aligned public health and sustainability advocates. Although not much difference is expected in 2015, the debate has activated political coalitions that could organize for the next DGA iteration in 2020.

Fourth, and perhaps most important, if the U.S. government adopts the DGAC’s reference to sustainability, it will sanction

and elevate discussion of sustainable diets. The U.S. government has been careful to relegate oversight of organic food to a government marketing program, which makes it safe for politicians to support organic as a matter of consumer choice without having to say whether it is healthier or has fewer environmental impacts than conventionally produced food. By acknowledging benefits of sustainability, the government would open itself up to greater demands for sustainability investments and would signal to consumers that such foods are preferred. Exponential growth in sales of organic, local, and sustainably harvested seafood suggests an appetite for sustainably produced food. People are motivated to change behavior for different reasons. Although shifting dietary choices for health reasons alone has not worked well, some people may be compelled to change diets to achieve sustainability goals.

In addition to the environmental impacts of food production, its economic sustainability must also be considered. The challenge is how to produce the most healthful foods in a way that sustains employment in the agricultural sector and minimizes adverse impacts on the environment. All major constituencies concerned with food security and health must wrestle with sustainability and dietary choices together. It is right and proper for the DGA process to lead the way. ■

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ACKNOWLEDGMENTS

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10.1126/science.aab2031

The martian lake chronicles

Curiosity reveals evidence for ancient lakes on Mars

By Marjorie A. Chan

Ray Bradbury's science-fictional archaeologist character, Hinkston, in *The Martian Chronicles*, thought: "Well, I think I'd rearrange the civilization on Mars so it resembled Earth more and more each day. If there was any way of reproducing every plant, every road, and every lake, and even an ocean, I'd do so. Then by some vast crowd hypnosis I'd convince everyone in a town this size that this really was Earth, not Mars at all." Hinkston may have his way without the need for hypnosis, because the latest discoveries on Mars geology, reported in this issue on page 177 by Grotzinger *et al.* (1), reveal records of lakes and other environments that remarkably resemble Earth. These findings provide "a good read" through the stratigraphic record of Mars, with tales of moving sand and pebble grains from ancient rivers to past lakes.

Grotzinger *et al.* chronicle the latest NASA Curiosity rover findings from the Mars Science Laboratory mission to find habitable environments. Curiosity is exploring a large pile of sedimentary rocks at Gale crater. Deciphering this geology is built on detective work, searching for clues and signs to tell us about past conditions, environments, and places where water could have harbored

life-forms [habitable environments (2)]. We rely heavily on Earth examples or terrestrial analogs that comprise the known baselines to help interpret the geological features of Mars.

At near-surface conditions, sediment grains are derived and recycled from preexisting rocks. Over time, these get buried and cemented, turning into hard sedimentary rocks. These rocks are often the most exciting because they record the history of a planet's surface and are most likely to preserve evidence of life such as fossils and microscopic biosignatures. Sedimentary rocks are layered, like pages in a book, starting at the bottom where the oldest layers were first laid down, and then go progressively upward through history to the youngest layers at the top.

Geologists read the rock record by interpreting all the stories of history—the past depositional environments, climate, and conditions that are preserved in layers or strata. But, like a long book that may have numerous pages, it helps to organize and divide a book into different chapters, each with a thematic essence. Similarly, to make sense of the rock layers, geologists find particular clues to lump the layers into logical, related packages. Bigger, thicker packages are named groups, with internally smaller subpackages called formations. Both groups and formations are stratigraphic units named for the place where they are first scientifically described. The NASA team describes and divides a 75-m-thick rock section into the Bradbury and Mount Sharp groups (see the figure).

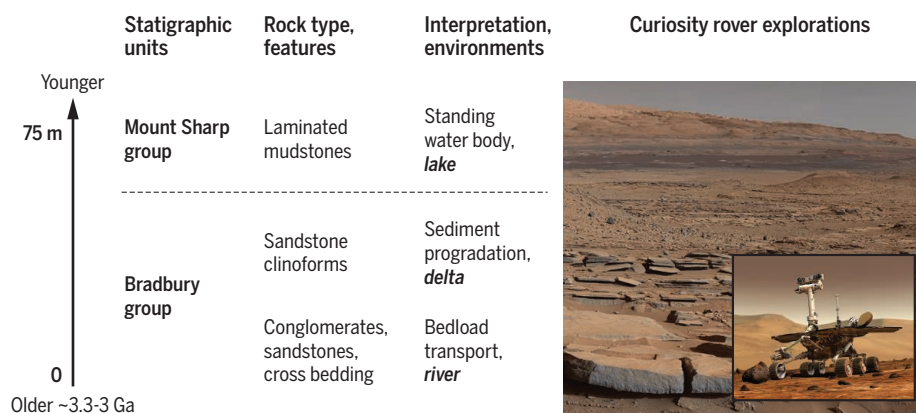
Within the sedimentary layers of these groups, they describe features that are diagnostic of three particular settings and conditions based on our knowledge of Earth analogs: Poorly sorted conglomerates and cross-bedded sandstones indicate bedload transport of sediment to comprise subaqueous (underwater) bedforms typical of rivers; clinoforms that are inclined bedsets in the shape of a flattened, stretched-out "s" suggest progressive accretion of sediment fed by rivers that enter a standing body of water like a lake, to comprise a transitional delta complex; and fine-grained sandstone and laminated mudstones suggest quieter water settings interpreted as a lake marginal to and intertonguing with the delta complex.

How did Mount Sharp, officially known as Aeolis Mons, rising up over 5 km from the crater floor, get inside Gale crater? At one time, many layers of sediment were deposited and laid down at the Gale crater site and, over time, erosion and exhumation (lifting to expose the rocks) occurred to generate the current configuration. Although climate conditions and the forces that sculpted and exposed the Bradbury and Mount Sharp groups will still be debated, these rock descriptions and interpretation set the stage for some of the first stories of martian lake records. The low depression of the Gale crater floor with its lake deposits suggest pooled and standing water for appreciable periods of time.

Although there is not yet definitive evidence for extraterrestrial life, the geologic results show that there were the key ingredients of water and places where water could have been accessible for microbial life to originate and evolve. Thus, the geology of Mars still holds the tantalizing possibility that extraterrestrial life might exist or have been preserved, because the evidence of water is so plentiful. On Earth, it is likely that any and all near-surface waters for the past ~3.5 billion years have been literally "contaminated" with some microbial life. Would Mars have had pure, abiotic waters? The more the geology looks like Earth, the more likely it seems that some life-form(s) could have developed in the martian waters. Life and water seem so intertwined that Bradbury's Hinkston character would have found his words prophetic: Mars seems to look more and more like Earth each day. ■

Department of Geology and Geophysics, University of Utah, Salt Lake City, Utah, UT 84112-0102, USA. E-mail: marjorie.chan@utah.edu

Geological martian chronicles



Interpreting the sedimentary record of Mars. The older Bradbury group rocks were studied in the rover's traverse from Bradbury Landing to Yellowknife Bay. The Bradbury Landing locality of the Curiosity rover was named after science-fiction author Ray Bradbury (3). The younger Mount Sharp group rocks were studied in the traverse from Yellowknife Bay to Pahrump Hills.

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10.1126/science.aad0902

BOOKS *et al.*

SCIENTIFIC APPROACHES

The domain of disciplines

Historical case studies offer insights into the future of interdisciplinary scholarship

By Yael Peled

“Interdisciplinarity,” like “multiculturalism,” is a notion of part-whole relations that is more often invoked than examined. Harvey J. Graff’s *Undisciplining Knowledge* is therefore a welcome addition to a surprisingly limited pool of existing titles on this topic. The book aims to counter the “accepted narrative” of interdisciplinarity in the modern research university as a linear and almost teleological process, often characterized by reductionism and little appreciation of history. In such narratives, the natural sciences are often taken as paradigmatic, and “oppositions and dichotomies stand in for critical definitions and in-depth, comparative research.”

Graff’s approach, by contrast, adopts an integrated historical, social, and contextual framework, which explores, in chronological order, the development of six pairs of interdisciplines: (i) genetic biology and sociology; (ii) the humanities and communication; (iii) social relations and operations research; (iv) cognitive science and new histories (contemporary approaches to historical processes that emphasize social and cultural factors); (v) materials science and cultural studies; and (vi) bioscience and literacy studies.

Graff offers the reader a complex tapestry of the history of interdisciplinary knowledge production and institutionalization. He discusses topics including the convergence of fields such as zoology, botany, bacteriology, and physiology that led to the emergence of biology; the impact of World War II and the Cold War on the field of operations research; and the diverse range of intramural (students, administrators, and research and development units) and extramural (governments, state institutions, private practitioners, corporations,

and lay citizens) end users of interdisciplinary research.

Graff’s detailed account of the six pairs also illuminates factors that are less visible but just as important in shaping interdisciplines. He discusses the role that technological advancement plays in shifting and sometimes overshadowing scientific questions (e.g., the effect of advances in computing on cognitive science) and the effect of power struggles among prominent re-

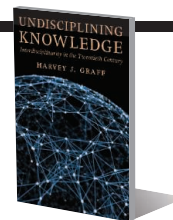


In *Undisciplining Knowledge*, Harvey Graff provides a new conceptual framework for considering interdisciplinarity.

searchers on the historical emergence of different interdisciplines. One particularly illuminating case study examines Talcott Parsons’s work at Harvard’s Department of Social Relations in the 1940s. Despite bringing together researchers from sociology, social and clinical psychology, and social anthropology in this newly established department, Parsons’s own work dominated the intellectual agenda, effectively rendering the “project” of social relations as an interdiscipline much less collaborative and synergetic than many had hoped.

The relationship between researchers and frameworks—and, more broadly, be-

Undisciplining Knowledge
Interdisciplinarity in the
Twentieth Century
Harvey J. Graff
Johns Hopkins University
Press, 2015. 341 pp.



tween fields and disciplines—is at the heart of interdisciplinary research and epistemology. And yet, as Graff repeatedly points out, it is very rarely discussed or debated. One of *Undisciplining Knowledge’s* major contributions is its careful and attentive mapping of the different paths to interdisciplinarity and the broader conclusions that may be drawn from the commonalities shared by the book’s 12 examples. Graff’s framework offers a fine-grained analysis of the substance and structure of interdisciplinarity, its continual interdependence on established disciplines, the factors that make some interdisciplines more successful than others, and the various models for interdisciplinarity. His specific pronouncements are certainly debatable, yet his proposed “taxonomy” provides a much-needed conceptual vocabulary to facilitate discussion.

Although heavily focused on the United States, *Undisciplining Knowledge* offers a detailed and illuminating account of the historical and intellectual forces that shaped interdisciplinarity in the 20th century and those that continue to do so today. These include the tension between specialization and generalization; the quest for epistemic unity and the danger of reductionism; the uneasy relationships between expertise and authority; and the complex links among research agendas, curriculum development, and expectations of practical applicability.

Graff emphasizes the dynamic interdependence between knowledge, scientific epistemologies, and (inter)disciplinarity, while remaining wary of proposing any simple definitions. Instead, he stresses the importance of egalitarian exchanges and the role of history and the humanities in the study of interdisciplinarity. Although *Undisciplining Knowledge* provides insightful answers to largely underexplored questions, its main contribution lies in refining and reframing these questions for the benefit of historians of science and interdisciplinary researchers.

The reviewer is at the Institute for Health and Social Policy and the Faculty of Law, McGill University, Montreal, Quebec H3A 1A3, Canada. E-mail: yael.peled@mcgill.ca

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RENEWABLE ENERGY

A bright future

The rise of solar energy and the path ahead for America's renewable energy sector

By **Amory B. Lovins**

In 1931, Thomas Edison remarked, "I would put my money on the sun and solar energy. What a source of energy!" He was right, but early. In 2014, only 0.5% of U.S. electricity was derived from solar energy. Yet even a little can be highly disruptive. For example, solar power production upended the business models and market value of major European utility companies before reaching even 5% of German power generation.

Solar power worldwide is scaling up faster than cellphones. Rooftop solar power has lately been adopted in Australia at an average per-capita rate 10 times that of California, reaching 90% of homes in some areas. And while solar power added 32% of new U.S. generating capacity in 2014, in 2013 alone, China added more photovoltaic (PV) capacity than the United States has added since Bell Laboratories unveiled the first modern solar cell in 1954.

PV panels are popping up everywhere—not just on roofs and roadsides but in inner cities, tribal lands, and military bases. A summary of what's happening and what it means has long been overdue, and Philip Warburg's readable and engaging *Harness the Sun* admirably fills this need. His more than 120 interviews vividly portray the diverse motives,

Solar farms can be built to accommodate livestock grazing and the habitats of native wildlife.

beliefs, styles, and methods of a host of entrepreneurs, activists, and ordinary citizens promoting American solar energy.

The fastest-growing U.S. energy source, solar power is rapidly creating income and jobs. The solar industry's pace drives and is driven by steeply falling prices (low prices make us buy more PVs, so they get cheaper, so we buy more, and so on). Spurred by German success, which inspired massive Chinese production, solar modules went on a price path akin to sneakers, falling more than 100-fold since 1975 and by 80% just in the past 5 years.

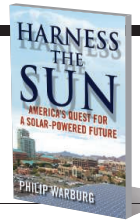
Warburg's lucid explanations of both PV and concentrating solar plants—whose mirrors or lenses focus solar light or heat onto a boiler—offer readers a broad context for understanding solar energy. He breaks down manufacturing processes, explains federal subsidies, explores ways to make solar panels affordable for renters and low-income communities, describes net metering (in which customers sell solar energy back to the grid), and insightfully dissects domestic solar politics and international trade disputes.

Some of the facts that Warburg presents are startling, like the EPA's finding of 5.5 trillion watts of solar-energy potential on U.S. lands believed to be contaminated with hazardous wastes ("brownfield sites"). But almost none of these facts are wrong. I had only a few quibbles. In chapter 5, Warburg refers to a study conducted by National Renewable Energy Laboratory (NREL) that claims that in order to supply 100% of America's power needs from the sun, solar installations would need to be erected on ~0.6 percent of the country's total land area. He argues, therefore, that "we shouldn't expect solar arrays on buildings, parking lots, and brownfields to satisfy all our electricity needs." However, the NREL study actually

Harness the Sun America's Quest for a Solar-Powered Future

Philip Warburg

Beacon Press, 2015. 251 pp.



excluded parking lots, which, by my calculation, could provide up to about half of U.S. electricity, especially at today's >21% module efficiency rather than the 13.5% that NREL assumed in 2008. Additionally, Warburg notes that ground-mounted PVs "blanket" up to half the land they occupy, but as he mentions later on, nearly all their total land use can be simultaneously used for livestock grazing, wildlife habitat, or other functions. And finally, in chapter 9, Warburg says that solar power costs more than gas power unless externalities are counted, but Bloomberg New Energy Finance, Lawrence Berkeley National Laboratory, and a half-dozen major financial houses have found that utility-scale solar, now averaging under a nickel per kilowatt-hour, generally beats gas plants' lifetime cost even without valuing carbon or the volatility of gas prices. It's true that this estimate includes a federal solar subsidy (which is set to fall from 30% to 10% after 2016), but permanent subsidies for nonrenewable energy sources are generally larger.

Warburg's graceful, diverse, wide-ranging, and well-balanced storytelling artfully fits a complex topic into 192 pages of text, which is easily digested in a sitting. That's a valuable service to a society rife with solar myths, many deliberately manufactured.

In a second edition, I'd suggest adding an index and three expansions. It would be worth discussing how the resilience of distributed renewables is vital not just for military bases but for all citizens. Of the billion-odd watts of rooftop solar power in New Jersey, for example, over 90% survived Superstorm Sandy, but not a solar watt worked without grid power. The utility companies that required solar power to depend on the grid are now moving to adopt industry consensus standards that let rooftop PVs work safely with or without the grid. Warburg fails to discuss flexible loads, distributed thermal storage, and other cheap substitutes for bulk electrical storage, whose supposed necessity is variable renewables' greatest myth. And finally, Warburg's sketch of how business models will transform to incorporate renewable energy resources just scratches the surface of a rich and important topic. But these are small gaps in what is ultimately an important and timely contribution to the public understanding of solar power.

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The reviewer is at Rocky Mountain Institute, Snowmass, CO 81654, USA. E-mail: ablovins@rmi.org

LETTERS

Edited by Jennifer Sills

Retraction

OUR REPORT “ULTRAHIGH magnetoresistance at room temperature in molecular wires” (1) presents measurements on one-dimensional molecular chains confined inside the nanochannels of zeolite L crystals. In these measurements, we observed signals that were interpreted as an exceptionally large (~1000%) response of the conductance through the molecular chains to an external magnetic field of a few millitesla. The explanation of the results was based on a room-temperature Pauli spin blockade effect, intrinsic to the hopping transport through the molecules. The observed magnetic field scale of a few millitesla could be explained by the typical magnitude of the random nuclear magnetic field in the molecular environment. The shape of the conductance versus magnetic field dependence was found to be in close agreement with similar curves observed in bulk organic semiconductors, in which the effect is referred to as “organic magnetoresistance” or “OMAR.” The exceptionally large effect in our case was ascribed to the one-dimensional nature of electron transport along the molecular chains.

In follow-up research by some of the co-authors, suspicion arose with regard to data collected by the first author Rabindra N. Mahato, which led to a thorough investigation by the co-authors. This investigation has revealed inappropriate data handling by Dr. Mahato, such that the experimental results are not accurately represented in the paper. This makes it, in our eyes, impossible to solidly underpin the conclusions made in the report. All co-authors have therefore concluded that the paper should be immediately retracted. Dr. Mahato has agreed to this Retraction.

R. N. Mahato,¹ H. Lülf,² M. H. Siekman,^{1,3} S. P. Kersten,⁴ P. A. Bobbert,⁴ M. P. de Jong,¹ L. De Cola,^{2,5} W. G. van der Wiel^{1*}

¹School of Physical Sciences, Jawaharlal Nehru University, New Delhi-110067, India. ²Institut de Science et d'Ingénierie Supramoléculaires (ISIS), Université de Strasbourg, Strasbourg, 67000, France.

³Transducers Science and Technology Group, MESA+ Institute for Nanotechnology, University of Twente, Enschede, 7500AE, Netherlands. ⁴Molecular Materials and Nanosystems, Department of Applied Physics, Eindhoven University of Technology, Eindhoven, 5600 MB, Netherlands. ⁵Karlsruher Institut für Technologie (KIT), Eggenstein-Leopoldshafen, D-76344, Germany.

*Corresponding author. E-mail: w.g.vanderwiel@utwente.nl

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In February 2015, Sierra Leone saw a resurgence in Ebola cases.

Speeding up epidemic emergency response

IN THEIR PERSPECTIVE “Ebola virus vaccines—preparing for the unexpected” (14 August, p. 693), H.-D. Klenk and S. Becker conclude that “many lives might have been saved if the phase 1 clinical studies in which the safety and immunogenicity of the vaccines against Ebola virus was assessed” had been completed earlier.

As contributors to and co-investigator of the study published in *The Lancet* (1), we have reflected on what additional measures could have been taken that would have facilitated an earlier implementation and conclusion of our trial.

(i) An earlier declaration of a Public Health Emergency of International Concern—June 2014 instead of 8 August—could have reduced the time by two months.

(ii) If all preclinical and clinical phase 1 studies had been concluded before the outbreak and a stockpile of vaccine of sufficient number of doses to carry out the phase 3 efficacy studies had been available before June 2014, this could have contributed another two months.

(iii) If Ebola-specific study protocols had been peer-reviewed and cleared in principle with participating partners, through improved international coordination of regulatory and ethical approval processes, this could potentially have reduced time by another month.

(iv) Because of the much higher case load in September 2014, compared with the case load in April 2015, the tail end of the study could possibly have been reduced by another month. We do acknowledge, however, there are operational limitations for appropriate inclusion and follow-up of trial participants.

In conclusion, in the case of the West African Ebola outbreak, phase 3 vaccine

studies could have been concluded in early 2015. This means that in future, analogous situations, an international blueprint for R&D preparedness and response could reduce the total time of implementing and concluding phase 3 vaccine trials during an outbreak. The World Health Organization and partners are currently in the process of developing such a blueprint. We hope the world will support such a framework for better international coordination of R&D for epidemic emergencies.

John-Arne Rottingen^{1,2,3} and Tore Godal^{4*}

¹Environmental Health and Infectious Disease Control, Norwegian Institute of Public Health, N-0403, Oslo, Norway. ²Department of Health Management and Health Economics, Faculty of Medicine, Institute of Health and Society, University of Oslo, Blindern, N-0317, Oslo, Norway. ³Department of Global Health and Population, Harvard School of Public Health, Boston, MA 02115, USA. ⁴Section for Global Initiatives, Ministry of Foreign Affairs, NO-0032, Oslo, Norway.

*Corresponding author. E-mail: Tore.Godal@mfa.no

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Free the tweet at scientific conferences

SOCIAL MEDIA ARE acknowledged more and more as tools to communicate science and influence policies. Meetings now have an active presence in social media, with specific hashtags developed to make each event identifiable and searchable. Twitter, for instance, has proved itself highly effective in disseminating the main ideas and results presented at a conference to both attendees and those who could not attend because of time constraints, economic reasons, or even physical or societal barriers. Such rapid communication has the potential to make science more inclusive, fostering collaboration and results. However, there is a new

and controversial trend to limit the free use of social media in scientific conferences.

At the 100th Anniversary Meeting of the Ecological Society of America celebrated in Baltimore in August (<http://esa.org/baltimore/>), with about 3800 attendees, the open use of Twitter (1) and photography (2) during talks was restricted unless permission from the organization or speakers had been granted for each specific talk. A few speakers during that meeting even used a “free to tweet” icon on their slides. Similar guidelines exist for numerous meetings organized by the Cold Spring Harbor Laboratory (3).

A restrictive policy regarding the use of social media in conferences clearly limits the impact of the communicated science and makes little sense in events with attendees in the thousands. We believe that results communicated in a public event should be viewed as public as if they were published in a journal. This would mean, unfortunately, that authors with confidential results or articles under embargo should keep them provisionally outside public view. With the generalized use of smartphones, tablets, and other communication devices in everyday life,

the scientific community should think strategically and clarify the role that social media should play in our activities.

Germán Orizaola^{1*} and Ana Elisa Valdés^{2,3}

¹Animal Ecology/ Department of Ecology and Genetics, Uppsala University, 75236, Uppsala, Sweden. ²Department of Physiological Botany, Uppsala BioCenter, Uppsala University, 75651, Uppsala, Sweden. ³Linnean Center for Plant Biology, 75651, Uppsala, Sweden.

*Corresponding author. E-mail: german.orizaola@ebc.uu.se

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TECHNICAL COMMENT ABSTRACTS

Comment on “Statistical binning enables an accurate coalescent-based estimation of the avian tree”

Liang Liu and Scott V. Edwards

Mirarab *et al.* (Research Article, 12

December 2014, p. 1250463) introduced

statistical binning to improve the signal in phylogenetic methods using the multispecies coalescent model. We show that all forms of binning—naïve, statistical, and weighted statistical—display poor performance and are statistically inconsistent in large regions of parameter space, unlike unbinned sequence data used with species tree methods.

Full text at <http://dx.doi.org/10.1126/science.aaa7343>

Response to Comment on “Statistical binning enables an accurate coalescent-based estimation of the avian tree”

Siavash Mirarab, Md. Shamsuzzoha

Bayzid, Bastien Boussau, Tandy Warnow

Liu and Edwards argue against the use of weighted statistical binning within a species tree estimation pipeline. However, we show that their mathematical argument does not apply to weighted statistical binning. Furthermore, their simulation study does not follow the recommended statistical binning protocol and has data of unknown origin that bias the results against weighted statistical binning.

Full text at <http://dx.doi.org/10.1126/science.aaa7719>

TECHNICAL COMMENT

AVIAN GENOMICS

Comment on “Statistical binning enables an accurate coalescent-based estimation of the avian tree”

Liang Liu¹ and Scott V. Edwards^{2*}

Mirarab *et al.* (Research Article, 12 December 2014, p. 1250463) introduced statistical binning to improve the signal in phylogenetic methods using the multispecies coalescent model. We show that all forms of binning—naïve, statistical, and weighted statistical—display poor performance and are statistically inconsistent in large regions of parameter space, unlike unbinned sequence data used with species tree methods.

Mirarab *et al.* introduced statistical binning as a method for improving the signal in species tree phylogenetic methods using the multispecies coalescent model and claimed that it can improve the accu-

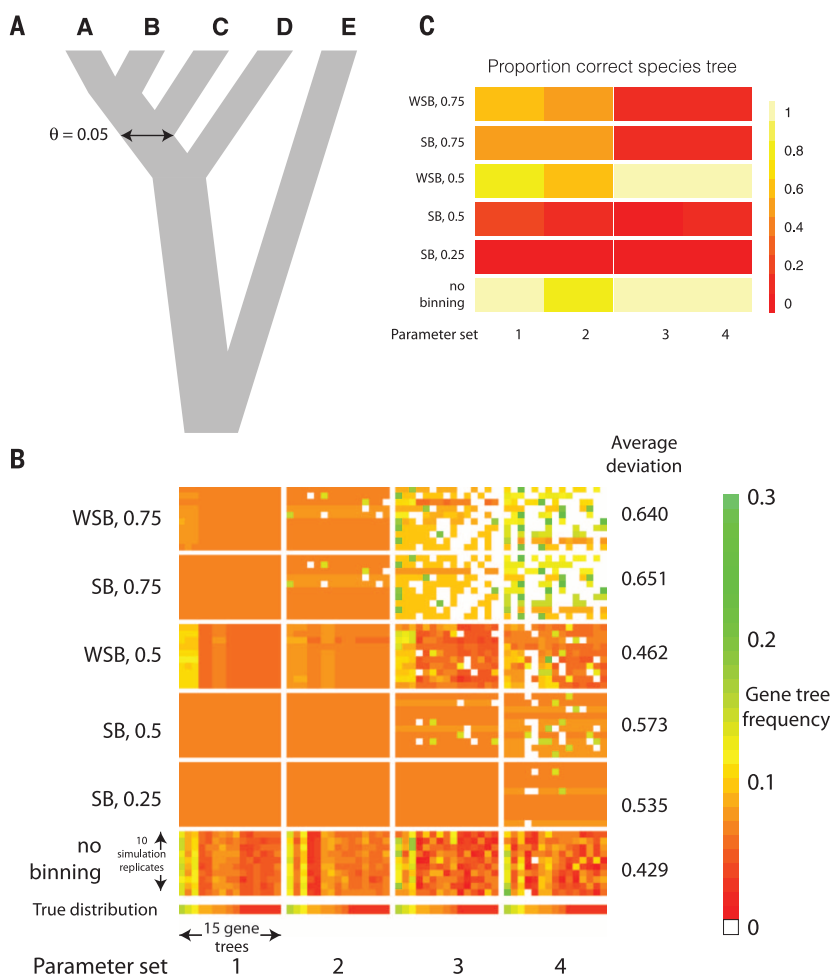
racy of coalescent-based estimation of species trees (1). Statistical binning is a method for signal augmentation in multilocus species tree reconstruction, designed to reduce gene tree estimation error by estimating supergene trees

from DNA sequences concatenated across the genes that do not conflict above an arbitrary bootstrap threshold. Mirarab *et al.* show a number of examples in which statistical binning appears to outperform unbinned species tree analysis as measured by the frequency of achieving accurate estimates of known phylogenies and species tree branch lengths. However, they explore a limited region of species tree parameter space that is favorable to binning analyses. We have recently shown (2) that naïve binning (NB), in which sequences are binned at random to create longer supergenes, without regard to their chromosomal location, exhibits poor performance in some regions of parameter space, because the method produces incorrect species trees with increasing certainty as the number of genes increases. Here, we show that statistical binning (SB), as well as its recent update, weighted statistical binning (WSB) (3), also exhibit inconsistent behavior, tending to distort the distribution of gene trees and converging

¹Department of Statistics, University of Georgia, Athens, GA 30602, USA. ²Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA.

*Corresponding author. E-mail: sedwards@fas.harvard.edu

Fig. 1. Binning simulation. Gene trees were simulated from a five-taxon species tree and then used to simulate DNA sequences using Seq-Gen (9) with the general time-reversible (GTR) + gamma model. We adopted the same GTR + gamma parameters used in (1) to simulate sequence data. We considered the following situations: number of genes = 100 or 1000, sequence length = 100 or 1000 base pairs (bp), and bootstrap threshold = 0.25, 0.50, or 0.75. The maximum likelihood (ML) and bootstrap gene trees were built for each gene using RAxML with the correct model. The estimated gene trees with bootstrap percentages were used without binning, or as input for statistical and weighted binning algorithms with varying thresholds, and the sequences within each bin were concatenated as a supergene according to the binning algorithm. Supergene trees were built using RAxML (10) with the correct model. Each simulation was repeated 10 times, but all the trends in our results were upheld with 100 replicates for each simulation. **(A)** The species tree used in the simulation is (((A:0.005, B:0.005):0.005, C:0.01):0.005, D:0.015):0.5, E:0.515) (branch lengths in substitutions per site), with the population size parameter $\theta = 0.05$. Binning is expected to perform worse for species trees in the anomaly zone (5, 6). **(B)** The true and estimated distributions of gene trees across simulations for six binning protocols, including no binning. Bootstrap thresholds are indicated. From left to right, four parameter sets, as follows: set 1: number of genes (n.g.) = 1000, sequence length (s.l.) = 1000 bp; set 2: n.g. = 1000, s.l. = 100 bp; set 3: n.g. = 100, s.l. = 1000 bp; set 4: n.g. = 100, s.l. = 100 bp. The 15 possible gene trees are represented along the x axis for each block of simulations. The y axis in each block represents 10 replicate simulations. Colors represent the values of probabilities, with white for gene trees not produced by the simulation. Flat distributions of gene trees are indicated by rows of the same color within blocks. The average deviation across the four parameter sets of the observed, reconstructed distribution of gene trees from the true distribution is indicated at left. For 100 replicates, including WSB0.25, these deviations are: no binning, 0.448; SB0.25, 0.543; WSB0.25, 0.449; SB0.50, 0.568; WSB0.50, 0.470; SB0.75, 0.678; WSB0.75, 0.667. These deviations were calculated as the sum of the absolute values of the differences between observed and true frequencies of gene trees. **(C)** A heat map for the proportion of the true species tree estimated among 10 replicates for each of four parameter sets, as in (B). Colors represent proportions. On the y axis are five different thresholds of statistical and weighted binning. Results for simulations with 100 replicates are similar, with the values for WSB0.25 varying from 1 (n.g. = 1000, s.l. = 100 bp) to 0.67 (n.g. = 100, s.l. = 100 bp).



to the wrong result under some parameter sets that otherwise fulfill the assumptions of the neutral multispecies coalescent model. Such convergence toward an incorrect result with increasing data set size is one prediction of an inconsistent method, yet species tree methods such as maximum pseudo-likelihood estimation of species trees (MP-EST) (4) have not exhibited this inconsistency under any parameter sets, including the anomaly zone, for analyses using unbinned loci.

SB yields a series of bins of roughly equal sizes, each of which includes a set of sequences consistent with a gene tree. Because the algorithm ensures that all supergene trees have frequency 1 in the binned data set, SB flattens the distribution of gene trees (3), thereby removing the coalescent signal maintained in individual gene trees and misleading downstream estimation of species trees for many parameter sets. The authors state that there is a high chance of binning genes with different histories (1), especially when the threshold is high and the bootstrap percentages on estimated gene trees are low. Such an outcome is likely when the internal branches in the species tree are short, a situation that generates short branches in gene trees. SB can produce highly biased distributions of supergene trees under some conditions (Fig. 1), and, just as full concatenation of alignments from genes with different

histories can positively mislead species tree estimation (5, 6), so can SB.

We used simulation to evaluate the performance of SB under a five-taxon (A to E) species tree that is close to but outside of the anomaly zone (Fig. 1A). As the root species E is fixed, there are 15 possible rooted gene trees. When $\theta = 0.05$ [high levels of incomplete lineage sorting (ILS)] and the bootstrap threshold is low (0.25), there is a high probability (>0.8) that SB will create a perfectly flat distribution of gene trees (Fig. 1B). When the threshold is high (0.75), bootstrap percentages on most gene trees are less than the threshold in our simulation, and most gene trees will be randomly distributed to different bins by the binning algorithm, similar to NB (7) (Fig. 1B). Under all sampling scenarios in our simulation, the gene tree distribution produced by SB, when combined with MP-EST, resulted in higher rates of estimating an incorrect species tree compared with no binning (Fig. 1C). This behavior is predicted by our sketch of the inconsistency of species tree methods under SB (Fig. 2).

WSB, in which each bin is assigned a weight equal to its size, has been proposed as a fix for the tendency of SB to flatten the distribution of gene trees (3). However, when WSB is applied to estimated gene trees, it may not be able to correct the flat distribution produced by SB. If the boot-

strap percentages on most estimated gene trees are less than the threshold, the binning algorithm will assign those gene trees at random to different bins, again resulting in flat distributions of gene trees under many parameter sets (Fig. 1B). Consistent with this tendency, we observe a much lower rate of correct species tree estimation across all simulations than without binning (Fig. 1C).

The empirical trees on which Mirarab *et al.* tested SB have many taxa, and the probability of generating two identical gene trees is very low, resulting in a true flat distribution of gene trees, leaving little opportunity for inconsistency of SB on species tree estimation. Mirarab *et al.* claim that binned trees are better estimated and more congruent with other analyses, but using concatenated trees as a benchmark is questionable. Nearly all of the species tree branches in unbinned analyses that Mirarab *et al.* claim are incorrect [figure 5 in (1)] differ nonsignificantly [as measured by bootstrap support (BS) less than 0.90] from binned analyses.

Outside of collecting more data, methods for signal augmentation in phylogenetics are extremely rare, with most methods instead focusing on improving model fit. We question the motivation behind SB: to improve the signal in gene trees and hence species trees. Rather, we suggest that the low signal often found in species trees is a real result that calls for more data collection—feasible even in analyses that purport to analyze whole genomes—or improved coalescent models, which binning is not. Binning (concatenation) might be used most profitably while taking genomic location into account, such as concatenating adjacent exons as frequently occurs in transcriptome data, which minimizes intralocus recombination, even though recombination is not a severe problem (8). Our demonstration that SB exhibits inconsistent behavior not observed in unbinned analyses and frequently distorts the distribution of estimated gene trees compels us to discourage its use. When support for a species tree is deemed too low, we suggest collecting more data and improving model fit rather than binning.

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A

Theorem 1: If the sequence length l is finite, there exists a species tree S with branch lengths L and a sufficiently small mutation rate μ such that $P(BS_{\max} > t) < \epsilon$ for every $\epsilon > 0$ and threshold t ($ML_{LB} < t < 1$).

Sketch of proof: Let x_i be the number of mutations occurring on branch i of an unrooted gene tree g . Under the substitution model, x_i has a Poisson distribution with mean $= b_i$ (the length of branch i). The probability that no mutation occurs on branch i is e^{-b_i} . Because the number of mutations on different branches (x_i) are independent of one another, the probability that no mutation occurs on all branches is $\prod_{i=1}^{2N-3} e^{-b_i}$. This probability converges to 1 as $b_i \rightarrow 0$ for $i = 1, \dots, 2N-3$. Because $b_i \rightarrow 0$ as the mutation rate $\mu \rightarrow 0$, the probability that no mutation occurs on all branches of the gene tree converges to 1 as $\mu \rightarrow 0$. When the alignments have no mutation, the EBPs on the ML gene tree equal the lower bounds LB of the EBPs. Because $t > ML_{LB}$, it follows that $P(BS_{\max} < t) > 1 - \epsilon$ for every $\epsilon > 0$. Thus, $P(BS_{\max} > t) < \epsilon$ for every $\epsilon > 0$.

B

Theorem 2: If the sequence length l is finite, there exists a species tree S with branch length L and population size θ , for which all supergene trees generated from unweighted or weighted binning converge in probability to the wrong trees as the number of genes goes to infinity.

Sketch of proof: The number of bins identified by unweighted or weighted binning equals the number of statistically different gene trees. As the number of taxa N is fixed, the number of bins is bounded. By Theorem 1, there exists a species tree S with branch lengths L and a sufficiently small mutation rate μ such that there is $(1 - \epsilon)$ proportion of gene trees, on which $BS_{\max}$ is less than the threshold t . These gene trees are randomly assigned to the bins identified by unweighted or weighted binning, and the probability distribution of the gene trees within each bin is identical to the probability distribution of gene trees under the multispecies coalescent model. Since ϵ can be arbitrarily small, we can find an $\epsilon > 0$ such that the effect of the remaining (ϵ) genes in estimating the supergene trees is negligible. In addition, because the number of bins is bounded, the number of genes within a bin goes to infinity as the number of genes increases. It follows from Theorem 1 in [5] that supergene tree SG_i built from the concatenated alignments converges to the wrong tree in probability as the number of genes goes to infinity.

Fig. 2. Inconsistency of binning. Let S be an N -taxon species tree with topology T and branch lengths L . Let SG be the ML supergene trees estimated from the sequences concatenated across genes within bins. Let t be the threshold defined in the binning algorithm for identifying statistically identical gene trees. $BS_{\max}$ denotes the maximum expected bootstrap percentage (EBP) on a ML gene tree. Let ML_{LB} be the maximum of the lower bounds LB of EBP. The lower bounds LB are achieved when no mutations are observed among all sequences. It is assumed that one allele is sampled from each species, so that the number of taxa in gene trees is equal to the number of taxa in the species tree. (A) Theorem 1 shows that the majority of the estimated gene trees generated from an anomalous species tree are poorly supported. Thus, given a threshold t , there exists an anomalous species tree such that the EBPs on the majority of gene trees are less than t . (B) Theorem 2 further shows that binning gene trees generated from an anomalous species tree positively misleads the estimation of supergene trees.

TECHNICAL RESPONSE

AVIAN GENOMICS

Response to Comment on “Statistical binning enables an accurate coalescent-based estimation of the avian tree”

Siavash Mirarab,^{1,2} Md. Shamsuzzoha Bayzid,¹ Bastien Boussau,³ Tandy Warnow^{1,4,*}

Liu and Edwards argue against the use of weighted statistical binning within a species tree estimation pipeline. However, we show that their mathematical argument does not apply to weighted statistical binning. Furthermore, their simulation study does not follow the recommended statistical binning protocol and has data of unknown origin that bias the results against weighted statistical binning.

In (1), we introduced statistical binning, a method to improve species tree estimation from multiple loci when true gene trees can differ from the species tree due to incomplete lineage sorting (ILS) (2). When ILS is present, unpartitioned concatenation using maximum

likelihood (ML) can be statistically inconsistent and fail to converge to the species tree as the number of loci increases (3). To address this challenge, statistically consistent coalescent-based “summary methods” have been developed [e.g., (4, 5)]. However, all current proofs of statistical consist-

ency for standard coalescent-based summary methods assume error-free gene trees. Furthermore, concatenated analyses can be more accurate than summary methods in the presence of substantial gene tree estimation error resulting from low phylogenetic signal (1, 5–11), a problem that confronted the Avian Phylogenomics Consortium (12). Because simulations showed that species trees computed with statistical binning followed by the summary method maximum pseudo-likelihood estimation of species trees (MP-EST) (4) “produced more accurate estimated species trees compared to MP-EST applied to unbinned gene data sets that have low phylogenetic signal” (12), the Avian Phylogenomics Consortium (which included Liu and Edwards) decided to use statistical binning with MP-EST to compute a coalescent-based avian species tree (12).

Because pipelines using statistical binning are not statistically consistent (11), we developed weighted statistical binning (WSB) (see Fig. 1) and proved that as both the number of loci and sequence length per locus increase, WSB followed

¹Department of Computer Science, University of Texas at Austin, Austin, TX, USA. ²Department of Electrical and Computer Engineering, University of California at San Diego, San Diego, CA, USA. ³Laboratoire de Biométrie Biologie Evolutive, Université de Lyon, France. ⁴Departments of Bioengineering and Computer Science, The University of Illinois at Urbana-Champaign, Urbana, IL, USA.

*Corresponding author. E-mail: warnow@illinois.edu

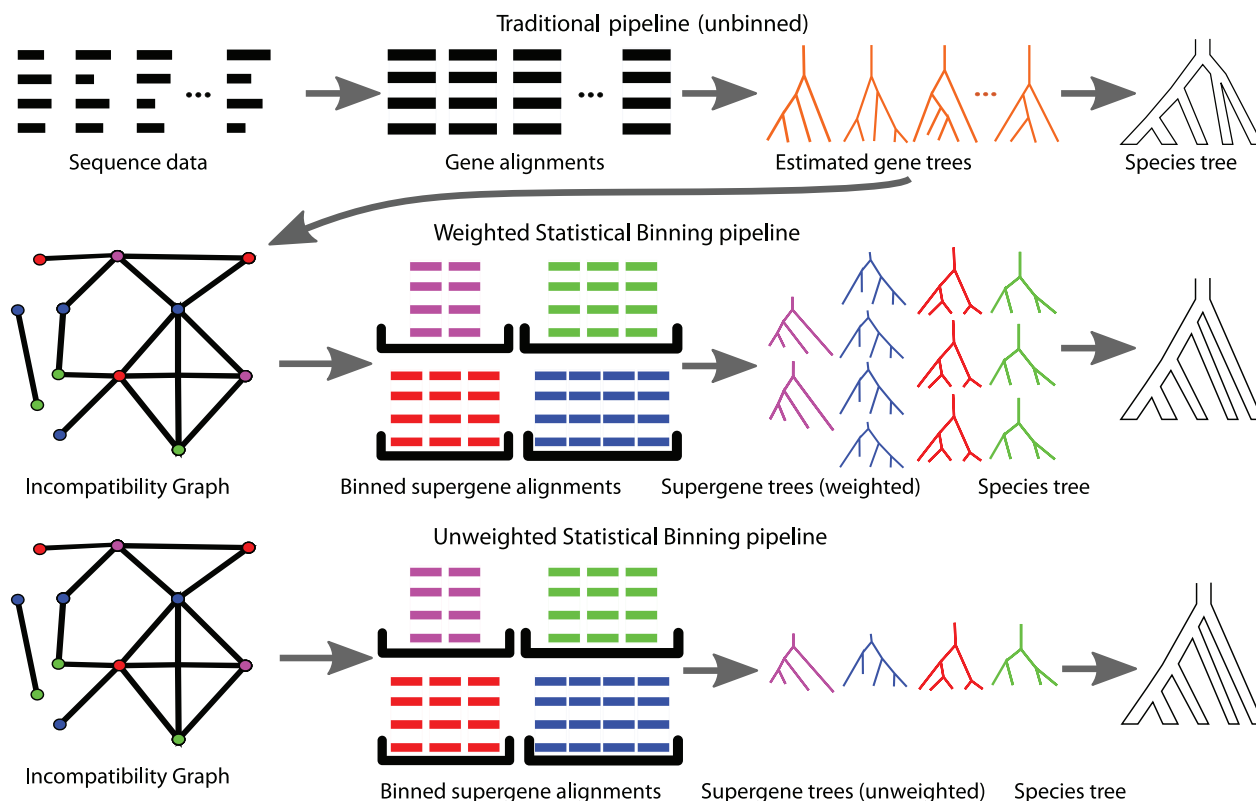


Fig. 1. Phylogenomic pipelines: unbinned (top), weighted statistical binning (middle), and unweighted statistical binning (bottom). Statistical binning divides the genes into bins that have no highly supported conflicts, estimates supergene trees on each bin, and then combines the supergene trees using the selected summary method. The WSB method differs from unweighted binning by replicating each supergene tree by the number of genes within its bin.

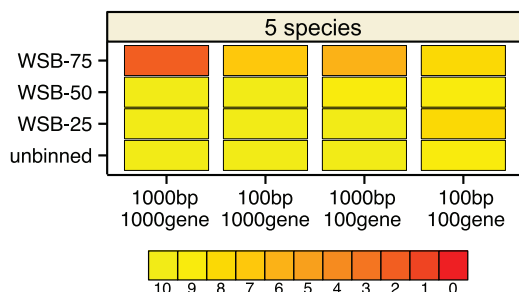
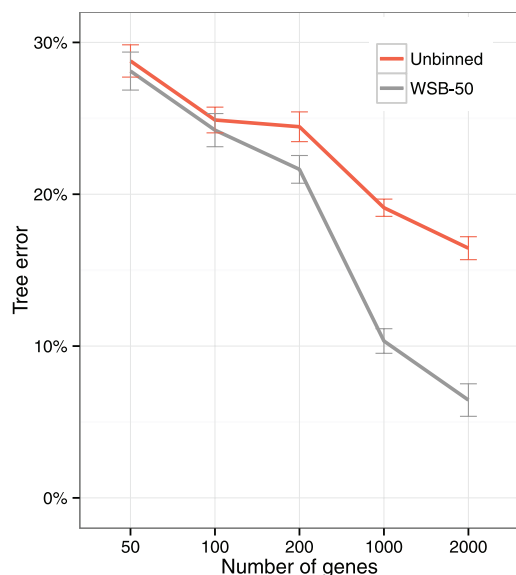
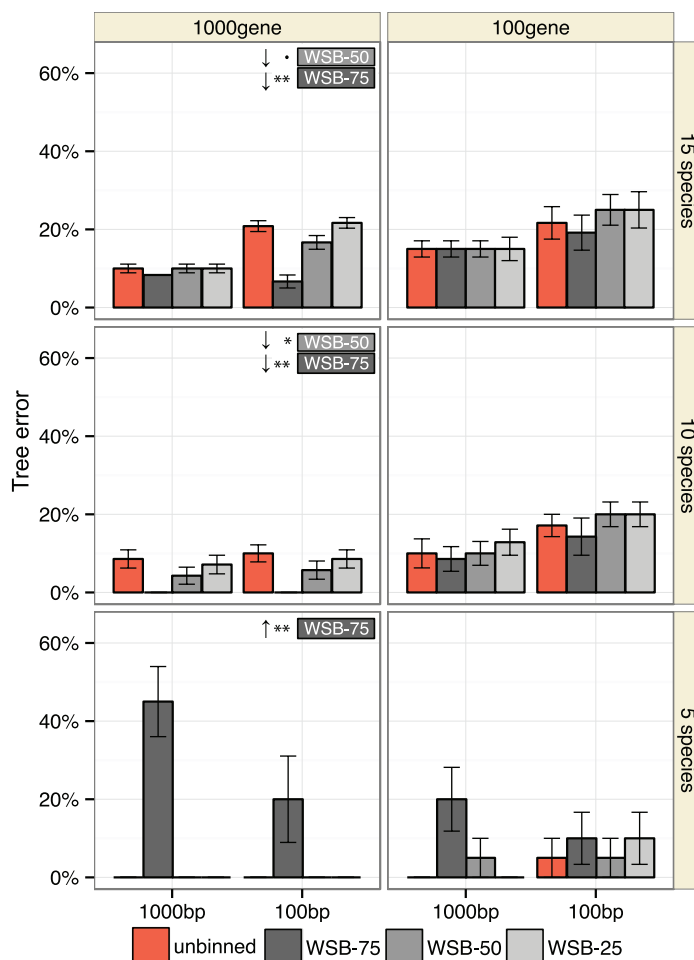
A Number of replicates with correct results**C** Simulations based on biological conditions**B** Simulations based on artificial conditions

Fig. 2. Simulation studies evaluating the impact of WSB on MP-EST analyses. (A) Tree error rates (percentage of missing branches) for species trees estimated with WSB (MLBS gene trees, fully partitioned ML analyses) and unbinned analyses on the five-species data sets studied by Liu and Edwards, and similar model conditions with 10 and 15 species, all with 1000 genes. Symbols: 1 indicates that using WSB increases species tree estimation

error; ↓ indicates that using WSB decreases error. (B) Number of replicates for which the true species tree is recovered on the five-species data sets studied by Liu and Edwards. (C) Results on simulated avian data sets with 1X branch lengths and 500-bp sequences per locus, based on the avian tree from (12). MP-EST analyses are based on multilocus bootstrapping, and unpartitioned ML analyses are used to compute supergene trees.

by any consistent summary method converges in probability to the true species tree (i.e., pipelines using WSB are statistically consistent) (17). However, it is not known whether WSB, fully partitioned concatenation, and standard summary methods are consistent or inconsistent, given bounded length sequences for each locus (6, 14).

Liu and Edwards attempt to prove that WSB will not converge in probability to the species tree when the number of loci increases but the sequence length per locus is bounded (13). Their mathematical argument does not apply to WSB, because it relies on a theorem in (3) that unpartitioned ML is inconsistent in the presence of ILS, whereas WSB uses fully partitioned ML to estimate supergene trees. Furthermore, the theorem in (3) cannot be applied to fully partitioned ML, and it is unknown whether fully partitioned ML is consistent in the presence of ILS (14). Therefore, we reject the statement in (13) that “all forms

of binning...are statistically inconsistent in large regions of parameter space.” Statistical inconsistency requires a mathematical proof, so Liu and Edwards have not established statistical inconsistency for WSB.

Liu and Edwards present a five-species simulation study in which they explored the impact of both weighted and unweighted statistical binning (13). However, many of the supergene alignments for parameter set 2 [1000 genes, 100 base pairs (bp)] contain sequences that did not come from any of the individual gene sequence alignments generated by Liu and Edwards. Importantly, these extraneous sequences in the supergene alignments reduce the accuracy of binned analyses but have no effect on unbinned analyses and thus introduce bias into the experiment.

Our analysis of their data (Fig. 2A) produced more accurate results for WSB than they reported. The differences on parameter set 2 are mostly due

to extraneous data in the supergene alignments they generated. However, we also determined that they used unpartitioned ML to compute supergene trees, which also reduced accuracy of WSB, whereas we used fully partitioned ML, as required for WSB’s theoretical guarantees.

In addition to the five-species data sets studied by Liu and Edwards, we explored similar model conditions with 10 and 15 species (Fig. 2B). Ten replicate data sets were generated under each model condition (number of taxa, number of genes, and sequence length). We evaluated the statistical significance of differences between methods, correcting for multiple tests (using false discovery rate correction, $n = 18$ statistical tests). There are no statistically significant differences for analyses with 100 genes (Fig. 2B). Results obtained on 1000 genes (Fig. 2B) show that WSB with bootstrap support (BS) threshold of 50% and 75% produced statistically significant reductions

in the species tree error rate for 15- and 10-species data sets ($P < 0.007$ for BS threshold of 75% on both 10- and 15-species data sets, $P = 0.044$ for BS threshold of 50% on 10-species data sets, and $P = 0.096$ for BS threshold of 50% on 15-species data sets). On the five-species, 1000-gene data sets (Fig. 2B), WSB and unbinned analyses were identical except when the BS threshold was 75%, which led to a statistically significant increase in the species tree estimation error ($P = 0.0069$). Thus, the effect of WSB depends on the model condition and BS threshold but was neutral to highly beneficial for all 10- and 15-taxon data sets that we analyzed.

The simulation condition explored by Liu and Edwards has a model species tree with only five species and very high ILS (i.e., the average topological distance between true gene trees and species trees is 82%), and evolves sequences under a strict molecular clock. The reduction in accuracy produced by using WSB on these data is consistent with a trend we reported in (11), where we observed that WSB can reduce accuracy on data sets with very high ILS and small numbers of species. However, for larger data sets, WSB almost always improved the accuracy of species tree topologies and branch lengths, and reduced

the incidence of strongly supported false positive branches (11). For example, WSB led to substantial improvements on the 48-taxon avian simulated data sets, which have a fairly high ILS level (average distance between true gene trees and species tree of 47%) (Fig. 2C).

Liu and Edwards only examined five-taxon data sets. Although performance on very small numbers of species is of interest for some analyses, the avian phylogenomics project (12) and many other phylogenomic data sets have substantially larger taxon sets. Thus, the research community needs species tree estimation methods that are highly accurate for large taxon data sets with gene tree estimation error. Although there is progress in the development of summary methods with good accuracy under these conditions (5), all current summary methods are affected by gene tree estimation error (1, 5–11). Hence, WSB provides a useful tool for species tree estimation in modern phylogenomic analysis.

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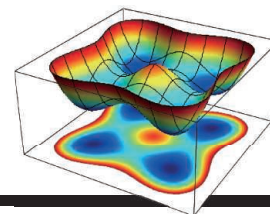
All simulated data, commands, and details about the simulation study are available at www.cs.utexas.edu/users/phylo/datasets/binning-response. Funding for this research was provided by the U.S. National Science Foundation (DBI-1461364) to T.W., by the Howard Hughes Medical Institute to S.M., and by the CNRS and Agence Nationale de la Recherche (ANR) through grant ANR-10-BINF-01-01 Ancestrisme to B.B.

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RESEARCH

1D topological edge states get chiral

Cheon et al., p. 182



IN SCIENCE JOURNALS

Edited by Nick Wigginton

VIROLOGY

Orchestrating a viral takeover

For some pathogenic viruses, outbreaks occur when a new viral strain emerges and displaces the endemic strain. How such a takeover occurs at a molecular level, however, remains an open question. Manokaran *et al.* examined one example, the emergence of a new clade of dengue virus (DENV) that caused an outbreak in Puerto Rico in 1994. The epidemic strain produced elevated amounts of subgenomic flavivirus RNA (sfRNA), a viral noncoding RNA, relative to amounts of genomic viral RNA. sfRNA bound to and inhibited TRIM25, a protein important for activating the host's antiviral response, and so by reducing host immunity was able to increase its own fitness. — KLM

Science, this issue p. 217



New dengue virus strains emerge by reducing host immunity

GEOMORPHOLOGY

The glacial way of wearing away

The rate at which glaciers erode landscapes is an important but poorly constrained relationship. Herman *et al.* tackle this issue by considering the Franz Josef alpine glacier in New Zealand. The amount of sediment piling up at the edge of the glacier provided erosion rates, whereas remote sensing allowed for simultaneous tracking of glacial motion. The result was a nonlinear relationship, suggesting that fast glaciers are much more effective at gouging landscapes. This could explain the paradox of why long-term erosion rates are so much lower in polar regions with more permanent glaciers. — BG

Science, this issue p. 193

FRUSTRATED MAGNETISM

Elucidating order within disorder

In some materials, the geometry of the crystal lattice gets in the way of magnetic ordering. Their spins, although magnetically interacting, remain seemingly disordered and form a so-called spin liquid. Paddison *et al.* propose a different model for the spin-liquid compound $\text{Gd}_3\text{Ga}_5\text{O}_{12}$. Neutron diffraction measurements and numerical techniques revealed that even though individual spins in this material were disordered, they formed 10-spin loops that were correlated with one another. The nature of this “hidden order” was such that it escaped direct detection by conventional techniques — JS

Science, this issue p. 179

SURFACE CHEMISTRY

Comparing active site reactivity

Noble metal nanoparticles often exhibit behaviors distinct from atomic and bulk versions of the same material. Gold and platinum dispersed on metal oxide supports, for example, show remarkable low-temperature reactivity for carbon monoxide (CO) oxidation by oxygen or water. Ding *et al.* used infrared spectroscopy to identify CO adsorbed on isolated platinum atoms or nanoparticles dispersed on zeolite and oxide supports. Temperature-programmed desorption studies showed that CO reacted at much lower temperatures when adsorbed on nanoparticles versus on isolated metal atoms. — PDS

Science, this issue p. 189

PLANT SCIENCE

Secondary cell walls built with speed

Plant cell walls provide the cellulose that is integral for wood, cotton fiber, and many biofuels. Cellulose is synthesized outside the cell membrane by cellulose synthase enzymes. Much of the secondary cell wall, responsible for the sturdiness of wood, is formed by xylem cells embedded in the core of the plant. Watanabe *et al.* leveraged ectopic expression to bring xylem-style cellulose synthase activity to the epidermal surface of the plant (see the Perspective by Schneider and Persson). Combining this improved accessibility with fluorescent tagging showed that secondary cell walls are built faster than primary cell walls, perhaps due to increased

velocity and density of cellulose synthase complexes. — PJH

Science, this issue p. 198,
see also p. 156

ASTROPHYSICS

Evidence of a universal physics of accretion

From nascent stars to super-massive black holes, most objects in the universe grow by the gradual accretion of material. One important signature of the accretion process is the amplitude of variability in the brightness of energy emitted from an astrophysical object. Scaringi *et al.* demonstrated that brightness variabilities of accreting white dwarfs and young stellar objects obey the same functional relationships with respect to brightness. Previously, these same functional relationships have been shown to hold for much larger objects, including supermassive black holes, implying a universality of the physics of accretion. — KVV

Science Adv. 10.1126/sciadv.1500686 (2015).

EDUCATION

A little bit of math goes a long way

Children's emerging language skills are supported when their caregivers read to them at home. Math skills, however, are often relegated to the schools. Berkowitz *et al.* developed a mobile-device app designed to help caregivers bring a little bit of math into the home. Improved math skills



Like reading, caregivers can help children improve their math skills away from school

were apparent within months for elementary school students. Improvements were most dramatic in families where the caregivers reported themselves to be anxious about math. — PJH

Science, this issue p. 196

ONCOLOGY

Is cancer immunotherapy a private affair?

Immune checkpoint blockade, a relatively new cancer treatment, substantially extends the survival of a subset of patients. Previous work has shown that patients whose tumors harbor the largest number of mutations—and thus produce a large number of “neo-antigens” recognized as foreign by the immune system—are most likely to benefit. Expanding on these earlier studies, Van Allen *et al.* studied over 100 patients with melanoma and found a similar correlation (see the Perspective by Gubin and Schreiber). There was no evidence, however, that specific neoantigen sequences were shared by patients who responded. — PAK

Science, this issue p. 207,
see also p. 158

TRANSPLANTATION

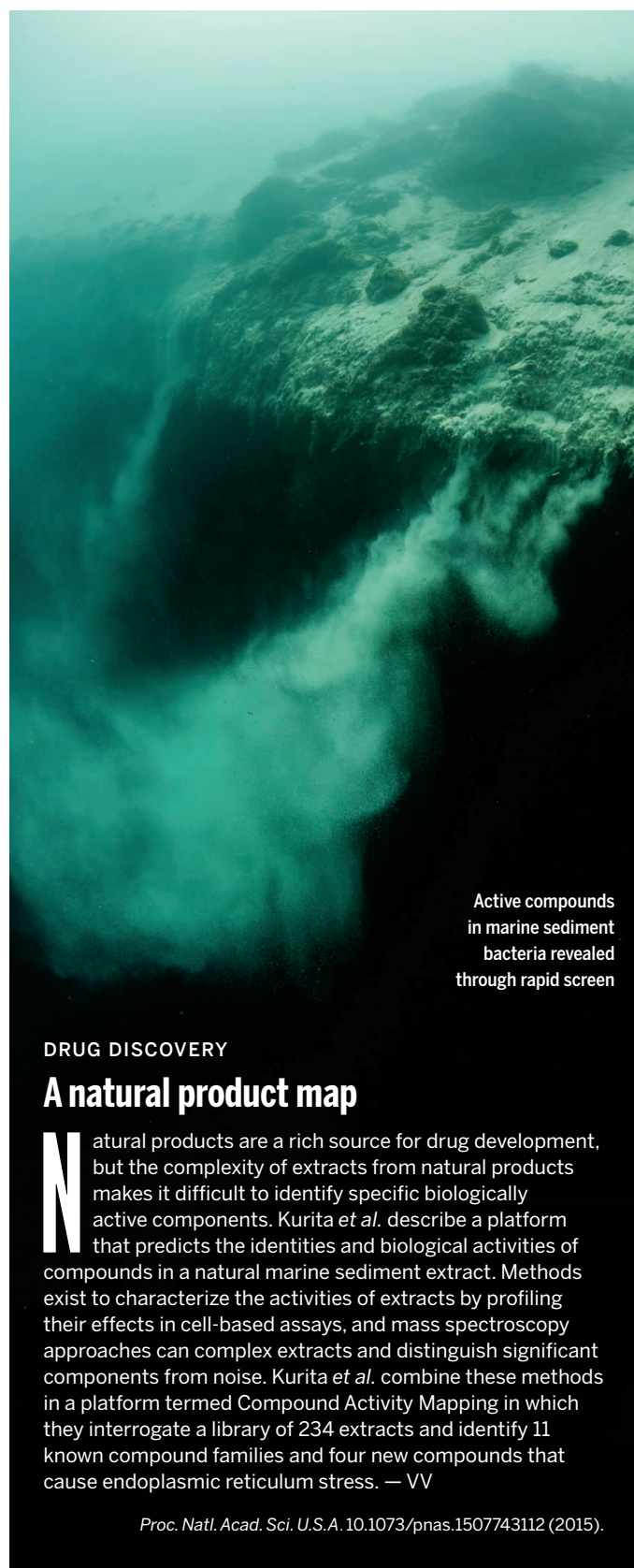
Blocking a fatal punch

Bone marrow transplantation replaces unhealthy bone marrow with that of a healthy donor. But donor-derived immune cells might recognize the transplant recipient as foreign and attack, resulting in graft-versus-host disease (GVHD). Zhang *et al.* report that blocking the suppression of tumorigenicity 2 protein (sST2), a serum marker for GVHD, with a neutralizing antibody reduces GVHD severity and mortality. The blockade decreased the production of proinflammatory cytokines and increased the frequency of anti-inflammatory molecules and cells while maintaining graft-versus-leukemia activity. Targeting sST2 might therefore decrease GVHD after bone marrow transplantation. — ACC

Sci. Transl. Med. 7, 308ra160 (2015).

IN OTHER JOURNALS

Edited by **Sacha Vignieri**
and **Jesse Smith**



Active compounds
in marine sediment
bacteria revealed
through rapid screen

DRUG DISCOVERY

A natural product map

Natural products are a rich source for drug development, but the complexity of extracts from natural products makes it difficult to identify specific biologically active components. Kurita *et al.* describe a platform that predicts the identities and biological activities of compounds in a natural marine sediment extract. Methods exist to characterize the activities of extracts by profiling their effects in cell-based assays, and mass spectroscopy approaches can complex extracts and distinguish significant components from noise. Kurita *et al.* combine these methods in a platform termed Compound Activity Mapping in which they interrogate a library of 234 extracts and identify 11 known compound families and four new compounds that cause endoplasmic reticulum stress. — VV

Proc. Natl. Acad. Sci. U.S.A. 10.1073/pnas.1507743112 (2015).

NEUROSCIENCE

Processing smells to elicit emotions

The amygdala is a brain region that plays an important role in emotions. Emotional processes in rodents depend heavily on olfaction. Mice transmit olfactory cues through two separate pathways, the main and the accessory olfactory systems. These pathways detect odors and nonvolatile chemosignals, respectively. Keshavarzi *et al.* studied the responses of principal neurons in the medial amygdala. These neurons received convergent inputs from both olfactory pathways. However, their synapses were located on different parts of the dendritic tree. Compared to main olfactory system inputs, accessory olfactory inputs projected more onto the distal dendritic arbor and showed a broader summation and a higher output gain. Neurons in the medial amygdala thus process main and accessory olfactory information differently. — PRS

J. Neurosci. **35**, 13020 (2015).

HUMAN GENETICS

Crowding is bad for your health

Pathological protein aggregates are known to be associated with many human neurodegenerative diseases, such as Alzheimer's. The tendency to form aggregates can come from mutations that cause either a gain or loss of function in a protein. However, the number of proteins that contribute to disease-associated aggregates has not been explored. De Baets *et al.* computationally explored databases of human genetic variation, including those from cancer studies, to determine the likelihood of protein aggregations. They found that disease-associated genes showed a higher number of mutations predicted to increase the likelihood of aggregation. These findings indicate that aggregation may more commonly contribute to disease

than previously thought. — LMZ

PLOS Comput. Biol. 10.1371/journal.pcbi.1004374 (2015).

INORGANIC CHEMISTRY

A magnesium catalyst to form B-N bonds

Boron and nitrogen pair up often in chemistry. Notable examples of that pairing include the classic ammonia borane adduct $\text{H}_3\text{N}-\text{BH}_3$, the hexagonal borazine, with alternating BH and NH groups; and the simple boron nitride BN, rivalling graphene in its exotic emerging two-dimensional properties. It may come as a surprise that not all B-N bonds are straightforward to make. Liptrot *et al.* report a magnesium catalyst that forms an underrepresented class of compounds linking amines to boron centers bearing two carbon or two oxygen bonds ($\text{R}_2\text{N}-\text{BR}'_2$) via release of H_2 . Specifically, the reaction is demonstrated for pinacolborane and 9-borabicyclononane with a variety of primary and secondary amines. Kinetics studies reveal intriguingly distinct mechanisms for each boron substrate. — JSY

Angew. Chem. Int. Ed. 10.1002/anie.201505949 (2015).

EDUCATION

First-hand accounts of diversity

Despite efforts to increase the number of minorities in science, underrepresentation persists. To investigate why, Gibau documented the experiences of minority graduate students participating in intervention programs. Data collected during individual interviews were analyzed to assess program effectiveness. These data exposed several challenges experienced by students, including withstanding the transition from historically black colleges and universities to predominantly white institutions, and the desire for authentic role models. Successes also were uncovered, specifically regarding



Protein abundance and cellular location change as rats age

AGING

Aging gracefully, one organ at a time

You're as old as you feel, goes the old adage, but what is aging at the cellular level? On *et al.* performed a proteomic and transcriptomic analysis of the brains and livers of 6-month-old young rats and 2-year-old rats.

More than 400 proteins changed in abundance between young and old animals, and more than 100 other proteins changed their cellular location, phosphorylation state, or splice form. Although some proteins and protein complexes were altered with age at similar rates, most changes were specific to one organ. These differences could be because of the very different life histories and functions of the two organs: Liver cells continually turn over and regenerate, whereas the brain mostly contains terminally differentiated postmitotic cells. — SMH

Cell Syst. 10.1016/j.cels.2015.08.012 (2015).

the peer interaction associated with some programs. Although these data are not generalizable, it is necessary to have a student evaluation of programs designed to benefit them. With further collection of similar data, program administrators can begin to measure progress toward increasing diversity in science. — MM

CBE Life Sci. Edu. 10.1187/cbe.14-06-0103 (2015).

APPLIED PHYSICS

A not-so-rigid way to get past the jam

When highly concentrated particles become jammed,

motion only happens in groups or through a cascading process. This rigidity transition, which for hard particles is accompanied by a transition to a solid-like state, is controlled by packing density. In some biological tissues, the packing fraction is almost always close to unity, but these materials still show glassy dynamics characteristic of the jammed state. Using the vertex model, Bi *et al.* show that the shape of the cell and the cell-to-cell adhesion correlate with whether the system is in a fluid or glassy state. They also identify a universal perimeter-to-area ratio where the jamming transition occurs. — MSL

Nat. Phys. 10.1038/nphys3471 (2015).

ALSO IN SCIENCE JOURNALS

Edited by Nick Wigginton

MARTIAN GEOLOGY

Ancient lake system at Gale crater

Since 2012, the Curiosity rover has been diligently studying rocky outcrops on Mars, looking for clues about past water, climate, and habitability. Grotzinger *et al.* describe the analysis of a huge section of sedimentary rocks near Gale crater, where Mount Sharp now stands (see the Perspective by Chan). The features within these sediments are reminiscent of delta, stream, and lake deposits on Earth. Although individual lakes were probably transient, it is likely that there was enough water to fill in low-lying depressions such as impact craters for up to 10,000 years. Wind-driven erosion removed many of these deposits, creating Mount Sharp. — NW

Science, this issue p. 177,
see also p. 167

NANOPARTICLES

Solutions for nanoparticle solutions

Nanoparticle interactions in solution affect their binding to biomolecules, their electronic properties, and their packing into larger crystals. However, the theories that describe larger colloidal particles fail for nanoparticles, because the interactions do not add together linearly. Nanoparticles also have complex shapes and are closer in size to the solvent molecules. Silvera Batista *et al.* review approaches that can treat the nonadditive nature of nanoparticle interactions, resulting in a more complete understanding of nanoparticles in solution. — PDS

Science, this issue p. 176

STRUCTURAL BIOLOGY

Opening up Vps34 protein complexes

During intracellular membrane trafficking, large protein complexes regulate and adapt the

activity of signal transducer enzymes such as the class III phosphatidylinositol 3-kinase Vps34. These large enzyme complexes are present in all eukaryotic cells, having widespread importance in neurodegeneration, aging, and cancer; however, a structural understanding has been lacking. Rostislavleva *et al.* provide atomic-resolution insights into the structures of the Vps34-containing protein complexes required for autophagy, endocytic sorting, and cytokinesis. The V-shaped complexes can undergo opening motions, which allows them to adapt to and phosphorylate membranes. — SMH

Science, this issue p. 178

HISTORY OF SCIENCE

Mendel, revisited

150 years ago, Gregor Mendel was getting ready to publish his work on dominant and recessive traits of pea varieties. The work was forgotten until the turn of the 20th century, when Mendel was recognized as the father of genetics. Some scientists concluded that Mendel's data were "too good to be true," but concerns over possible data manipulation have proved to be unfounded. Yet as Radick discusses in a Perspective, the debate over potential fraud has dominated critical discussions of Mendel's work. Broader critiques of Mendel's legacy should, for example, take a closer look at his binary inheritance categories, which do not allow for the diversity of inherited characters. — JFU

Science, this issue p. 159

CANCER

Killing cancer cells with aggregates

The drug verteporfin, which is used clinically to enhance phototherapy, may also be useful as a cancer chemotherapeutic. Zhang *et al.* show that verteporfin triggered the accumulation of protein oligomers that selectively killed

colorectal cancer cells in mice and in cells cultured in hypoxic and nutrient-deprived conditions (see the Focus by Avril and Chevet). Normal cells in culture and in tumor-adjacent tissue sections from mice cleared these aggregates through autophagy and survived. Verteporfin thus produces tumor-selective proteotoxicity, which may be a useful therapeutic for patients with solid tumors. — LKF

Sci. Signal. **8**, ra98 and fs17 (2015).

TOPOLOGICAL MATTER

Handedness at the edge of a line

Topological insulators are characterized by conducting boundary states. For those existing as two-dimensional (2D) materials, the boundaries are lines, the edge currents are 1D, and their two spin components flow in opposite directions. To address whether this handedness also applies to the edge states of 1D topological systems, Cheon *et al.* deposited indium atoms on the surface of silicon, where the atoms formed wires consisting of double zigzag chains. The chains underwent distortions that caused topological edge states called solitons to appear under certain conditions. The solitons came in three flavors, two of which had a definite handedness. — JS

Science, this issue p. 182

SURFACE CHEMISTRY

Accounting for surface coordination

The exploration of heterogeneous catalysts using first-principles calculations can be daunting because the large number of atoms and possible surface geometries. Calle-Vallejo *et al.* describe a simpler metric for assessing optimal reactivity: a weighted average of surface coordination that includes second-nearest neighbors (see the Perspective by Stephens *et al.*).

The calculations identified three approaches for introducing cavity sites into the platinum(111) surface to improve its performance for the oxygen reduction reaction used in fuel cells. — PDS

Science, this issue p. 185,
see also p. 164

ONCOGENE SIGNALING

Cancer as a case of uncontrolled traffic

Healthy cells are like skilled air traffic controllers. They continually move proteins to and from the cellular destinations where they are needed, usually without mishap, through an elaborate system of endomembranes. Wheeler *et al.* show that a glitch in the traffic control system can help propel a cell toward malignancy (see the Perspective by Ferguson). RAB35, a protein previously implicated in endomembrane trafficking, is a key regulator of a well-known oncogenic signaling pathway. Mutations in RAB35 found in certain human tumors aberrantly activate this pathway and cause mislocalization of a factor that promotes cell growth. — PAK

Science, this issue p. 211,
see also p. 162

PLANT SCIENCE

Striga uses a hypersensitive receptor

The strigolactone hormones govern plant growth and development. A nasty parasitic weed, *Striga*, senses traces of these hormones to identify its targets. After functionally characterizing several strigolactone receptors from *Striga*, Toh *et al.* solved the crystal structure of one that is especially sensitive. The structure shows an unexpectedly large ligand-binding pocket, which may explain how *Striga* manages to sense picomolar concentrations of the range of strigolactones. — PJH

Science, this issue p. 203

REVIEW SUMMARY

NANOPARTICLES

Nonadditivity of nanoparticle interactions

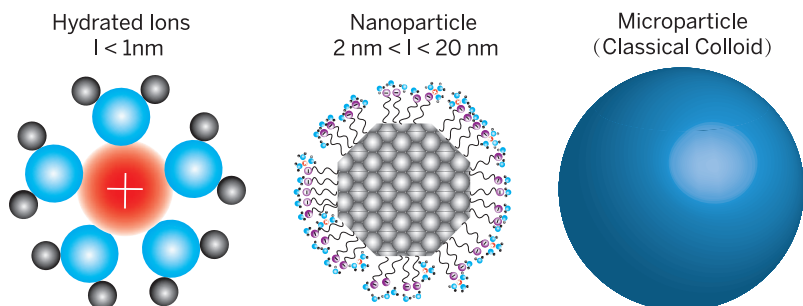
Carlos A. Silvera Batista, Ronald G. Larson,* Nicholas A. Kotov*

BACKGROUND: Interactions between inorganic nanoparticles (NPs) are central to a wide spectrum of physical, chemical, and biological phenomena. An understanding of these interactions is essential for technological implementation of nanoscale synthesis and engineering of self-organized NP superstructures with various dimensionalities, collective properties at the nanoscale, and predictive biological responses to NPs. However, the quantitative description of NP forces encounters many obstacles not present, or not as severe, for microsize particles (μ Ps). These difficulties are revealed in multiple experimental observations that are, unfortunately, not fully recognized as of yet. Inconsistencies in the accounting of NP interactions are observed across all material platforms that include ceramic, semiconductor, and metallic NPs; crystalline and amorphous NPs; as well as dispersions of inorganic, organic, and biological nanomaterials. Such systematic deviations of theoretical predictions from reality point to the generality of such phenomena for nanoscale matter. Here we analyze the sources of these inconsistencies and chart a course for future research that might overcome these challenges.

ADVANCES: Nanoparticle interactions are often described by classical colloidal theories developed for μ Ps. However, several foundational assumptions of these theories, while tolerable for microscale dispersions, fail for NPs. For example, the sizes of ions, solvent molecules, and NPs can be within one order of magnitude of each other, which inherently disallows continuum approximations. Fluctuations of ionic atmospheres, ion-specific effects, enhanced NP anisotropy, and multiscale collective effects become essential for accu-

rate accounting of NP interactions. The nonuniformity of the stabilizing layer adds another essential contradistinction.

When the particle size becomes smaller than a few tens of nanometers and the gaps between particles become smaller than a few nanometers, nonadditivity of electrostatic (V_{el}), van der Waals (V_{vdW}), hydrophobic (V_{hph}), and



Schematics of hydrated ions, a NP, and a μ P, demonstrating the structural uniqueness and discreteness of NPs. Nonadditivity of NP interactions stems from the size similarity of reconfigurable structural elements of NPs (i.e., surface ligands, ionic atmosphere, adsorbed molecules, etc.) and the surrounding media, leading to their strongly coupled dynamics. The high polarizability and faceting that are typical of NPs—as well as collective multibody effects at atomic, molecular, and nanometer scales—lead to the enhancement of nonadditivity and result in interdependence of electrostatic, van der Waals, hydrophobic, and other forces.

other potentials (V') emerges. In fact, it becomes impossible to cleanly decompose the potential of mean force (PMF) for the interaction of two NPs into separate additive contributions from these interactions—as in, e.g., classical Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [$V(r) = V_{el}(r) + V_{vdW}(r) + V_{hph}(r) + V'(r)$ —due to the coupled structural dynamics of neighboring NPs and surrounding media. Experimentally, the nonadditivity of NP interactions was observed long ago as an unusually high colloidal stability of NP dispersions, defying all reasonable predictions based on DLVO and other classical theories. It also manifests itself in paradoxical phase behavior, self-assembly into sophisticated superstructures, complex collective behavior, enigmatic toxicology, and protein-mimetic behavior of inorganic NPs. Molecular dynamics sim-

ulations of PMFs computed for NP pairs confirm the nonadditivity of van der Waals and electrostatic interactions for nanometer-scale separations.

OUTLOOK: Further work in the field of NP interactions should perhaps embrace NPs as

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strongly correlated reconfigurable systems with diverse physical elements and multiscale coupling processes, which will require new experimental and theoretical tools. Meanwhile, several heuristic rules identified in this Review can be helpful for discriminating between the systems in which mean-field theories can and cannot be applied. These precepts can guide qualitative thinking about NP interactions, stimulate further research into NP interactions, and aid in their design for applications.

Though it is the crux of nonadditivity, the similarity in size between the ions and molecules composing the solvent medium and the NP offers a silver lining: it makes atomic simulations of their interactions increasingly practical as computer speed increases. The direct determination of the PMF by atomistic simulation bypasses the enumeration of individual forces and therefore resolves the nonadditivity problem. In fact, NPs present a favorable system for atomistic simulations because solid inorganic cores have many fewer degrees of freedom than flexible organic chains. Improvements in force fields are necessary to

adequately account for intermolecular interactions, entropic contributions, dispersion interactions between atoms, high polarizability of inorganic materials, and quantum confinement effects.

Evolving experimental tools that can accurately examine interactions at the nanoscale should help to validate the simulations and stimulate improvement of relevant force fields. In fact, new opportunities for better understanding of the electronic origin of classical interactions are likely as the rapidly improving capabilities in synthesis, simulations, and imaging converge at the scale of NPs. ■

The list of author affiliations is available in the full article online.

*Corresponding author. E-mail: rlarson@umich.edu (R.G.L.);

kotov@umich.edu (N.A.K.)

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REVIEW

NANOPARTICLES

Nonadditivity of nanoparticle interactions

Carlos A. Silvera Batista,^{1,2} Ronald G. Larson,^{1,2,3,*} Nicholas A. Kotov^{1,2,3,4,†}

Understanding interactions between inorganic nanoparticles (NPs) is central to comprehension of self-organization processes and a wide spectrum of physical, chemical, and biological phenomena. However, quantitative description of the interparticle forces is complicated by many obstacles that are not present, or not as severe, for microsize particles (μ Ps). Here we analyze the sources of these difficulties and chart a course for future research. Such difficulties can be traced to the increased importance of discreteness and fluctuations around NPs (relative to μ Ps) and to multiscale collective effects. Although these problems can be partially overcome by modifying classical theories for colloidal interactions, such an approach fails to manage the nonadditivity of electrostatic, van der Waals, hydrophobic, and other interactions at the nanoscale. Several heuristic rules identified here can be helpful for discriminating between additive and nonadditive nanoscale systems. Further work on NP interactions would benefit from embracing NPs as strongly correlated reconfigurable systems with diverse physical elements and multiscale coupling processes, which will require new experimental and theoretical tools. Meanwhile, the similarity between the size of medium constituents and NPs makes atomic simulations of their interactions increasingly practical. Evolving experimental tools can stimulate improvement of existing force fields. New scientific opportunities for a better understanding of the electronic origin of classical interactions are converging at the scale of NPs.

Over the past 20 years, there has been rapid progress toward the synthesis of nanoparticles (NPs) and the understanding of their distinct size- and shape-dependent physical phenomena. Preparation of NP dispersions has led to promising new materials for electronics, optics, energy storage, catalysis, medicine, and other technologies (1). Moreover, the ability of NPs to associate also leads to self-organization into superlattices (2–5) and supraparticular assemblies of varying dimensions (6, 7). Multiparticle interactions are also responsible for the emergence of collective properties that are not present in individual NPs and encompass a wide spectrum of physical and chemical phenomena exemplified by Anderson excited states (8), multiparticle plasmonic resonances (9), collective Coulomb blockades (10), and others. Advancing these areas of knowledge and related technologies will require a detailed understanding of inter-NP interactions. Despite contributions from several disciplines and numerous authors, quantitative or even qualitative accounting of NP interactions is rarely achieved. The forces between NPs are largely the same as those between microsize particles (μ Ps). However, applying μ P theories (11–13) and equations to NPs is problematic. The purpose

of this Review is to (i) explain the source of these problems; (ii) organize diverse experimental, theoretical, and computational data into a consistent framework capable of describing interparticle interactions and assembly phenomena at the nanoscale; and (iii) chart a course for future studies that might overcome these obstacles.

What is a nanoparticle?

Within the framework of this Review, we define a NP as an inorganic particle, between 1 and 20 nm in diameter, together with a surrounding interfacial layer. The conclusions made for particles within this size range are valid for 100-nm particles that represent a commonly agreed dimensional threshold for nanoscale materials. However, the 1- to 20-nm size range provides the most vivid manifestations of quantum confinement, plasmonic effects, and other phenomena distinct to nanoscale materials. Yet the most severe problems in terms of agreement with theory tend to occur at this length scale, due to the convergence of the size of the constituents to within one order of magnitude of the particle size. Several characteristic distances describing interparticle interactions that fall into this range are discussed below.

We include the interfacial layer in the NP definition because measurements of properties of quantum dots, nanocrystals, nanowires, nanotubes, nanoplates, etc., all indicate that the interfacial layer is an integral part of nanoscale matter, fundamentally affecting its properties. The interfacial layer typically consists of organic molecules known as stabilizers, capping and surface ligands, or passivating agents. The inorganic part of the NPs (the

core) is often (mono)crystalline; hence, the term “nanocrystal” is sometimes used interchangeably with “nanoparticle.” Most of the considerations below are equally applicable to particles with cores that are not crystalline or polycrystalline, as long as they conform to the definition above. In fact, most, although not all, of the findings discussed in this paper are applicable to nanoscale particles of organic and/or biological materials (14, 15). Here we will primarily focus on particles with inorganic cores.

Breakdown of common assumptions for particle interactions at the nanoscale

Both NPs and μ Ps are often similarly treated as classical colloids. Both types of particles often contain an inorganic core coated with a layer of surfactant (Fig. 1). Another commonality is that electrostatic and van der Waals (vdW) interactions are the two main forces between both μ Ps and NPs.

However, essential distinctions between NPs and μ Ps result from their size difference of one to five orders of magnitude (Fig. 1A). At least four key assumptions of classical theories, such as the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, are often valid at macro- and microdimensions but are no longer applicable when particles reach nanoscale size. The failures of the first two of these assumptions for NPs are the most difficult to overcome.

The first assumption is that solvent molecules and solvated ions are negligibly small compared with the dispersed particles. Contrary to such a model, the dimensions of the 1- to 20-nm NPs are comparable to the dimensions of solvent molecules, solvated ions, and other components of the solution, requiring consideration of the structural discreteness within the NPs and the surrounding medium.

The second assumption is that the total potential is a sum of multiple independent repulsive and attractive components (12, 16)

$$V(r) = V_{\text{el}} + V_{\text{vdW}} + V' \quad (1)$$

where r is the center-to-center distance between the particles and V_{el} , V_{vdW} , and V' are distance-dependent potentials for electrostatic, vdW, and other interactions, respectively. Eq. 1 may also be referred to as the additivity assumption (17, 18); it fails as the size of the particles and the distances between them reach nanoscale dimensions. NPs are often made from metals (such as gold, silver, platinum, nickel, and cobalt) or semiconductors (such as PbSe, PbS, and CdTe) with high polarizability that increases the coupling between different interactions, exacerbating the problem.

The third assumption is that the media outside and inside of the particle are uniform continua. Although this is related to the first assumption, it is treated separately in the original theories, which prompted us to keep these two assumptions distinct. Additionally, the first assumption refers primarily to the bulk state of the solvent, whereas the third assumption refers primarily to the interface between the NP and the surroundings. The breakdown of the third assumption for NPs is associated, among other

¹Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109, USA. ²BioInterfaces Institute, University of Michigan, Ann Arbor, MI 48109, USA.

³Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA. ⁴Department of Materials Science and Engineering, University of Michigan, Ann Arbor, MI 48109, USA.

*Corresponding author. E-mail: rlarson@umich.edu (R.G.L.); kotov@umich.edu (N.A.K.)

factors, with the interfacial layer, which has a thickness comparable to or sometimes even greater than the diameter of the NP cores. Thus, the thickness of the interfacial layer cannot be neglected, and both NPs and their surroundings can no longer be considered uniform continua. Another consequence of its breakdown is that the classical continuous dielectric function (ϵ) must be replaced with local atomic polarizability. By introducing image charges, advanced versions of DLVO and other colloidal theories can partially account for differences in ϵ between core, surface layer, and media. However, this approach also fails when the system displays molecular and nanoscale heterogeneity, including discreteness of ionic charge that cannot be ignored for NPs and their self-assembly processes (19).

The fourth assumption typical of the classical theory of colloids is that particles have simple shapes, typically spherical and less often cylindrical or ellipsoidal. The crystallinity of a NP core, on the other hand, leads to a great expansion of the palette of particle shapes: rods, dumbbells, cubes, hexagons, tetrahedrons, octahedrons, concave rhombic dodecahedra, tetrahexahedra, and others (Fig. 1, B to G) (20). Even NPs that are considered to be spherical are often actually prolate in shape, with an aspect ratio of 1.1 to 1.2 (21), and are also often faceted (20). Although the nonspherical shapes and interaction potentials can be incorporated into the classical colloidal theories, asymmetry of the shape may also originate from spontaneous unevenness of the surface layer, as was demonstrated for both gold and silica NPs (Figs. 1, B and C) (22, 23). NPs with a thick stabilizer layer (Fig. 1A) can essentially change shape depending on the local environment. Such dynamic reconfiguration of particles

is difficult to account for in the traditional models. Also, the presence of sharp apices in tetrahedrons, pyramids, stars, and other particles creates singularity points that affect both the physics and chemistry of NPs. These singularity points are well known in plasmonics as so called “hot spots,” but they are largely neglected in the consideration of intermolecular forces.

Although these assumptions are not always accurate for colloidal systems, many μ P dispersions are lenient enough to neglect their failures, but the same is not true for NP dispersions.

Can DLVO theory be adapted to NPs?

The interactions between μ Ps are typically described by the DLVO theory. In its original formulation, DLVO theory considers only the two contributions V_{el} and V_{vdW} (24, 25). The mean-field Poisson-Boltzmann (PB) formalism is typically used to obtain V_{el} , with the Debye-Hückel approximation yielding an analytical solution for small ionic strengths (I) not exceeding 0.01 M for 1:1 electrolytes and 0.0001 M for ions with high ion charge (z_i) (12). V_{vdW} is commonly calculated using the Hamaker theory (26) simplified by the Derjaguin approximation (24). This potential describes London dispersion interactions that are equated with vdW interactions within classical DLVO formalism. A potential of mean force (PMF) between two particles is calculated according to Eq. 1 as $V(r) = V_{el} + V_{vdW}$.

Over the past 50 years, much scientific work has been devoted to extending and improving DLVO theory to allow for more accurate equations for V_{el} and V_{vdW} , as well as wider ranges of I and z_i . Later versions of DLVO and the Sogami-Ise theory (27) give much improved predictions for counterions with $z_i > 1$, asymmetric electro-

lytes, and nonspherical particles. These theories may also include other types of interactions, allow a finite size of hydrated counterions, and account for discontinuities of the dielectric constant at interfaces (28, 29). However, because of the conceptual problems noted above, even adaptations of many elegant theories developed for μ Ps are unlikely to have general applicability for NPs in the way that DLVO theory is applicable to microscale colloids. In particular, when particle size is reduced to the nanoscale, one needs to consider the finite size of solvated ions that are treated as point particles in DLVO theory and its many extensions. The diameters of common ions with their hydration shells encountered in NP dispersions are, for instance, 0.3 to 0.64, 0.45 to 0.47, 0.46 to 0.5, 0.40 to 0.6, 0.34 to 0.8, and 0.7 to 1.1 nm for Cl^- , Na^+ , Cd^{2+} , Ca^{2+} , Mg^{2+} , and CO_3^{2-} , respectively (30–32); these values are comparable to the diameters of many small NPs. Moreover, two interacting NPs in typical assemblies often have a gap (d) as narrow as 1 to 2 nm between them (Fig. 1, D to G). Thus, even for the smallest ions and simplest interfacial layers, theoretical descriptions that ignore their finite dimensions cannot be applied to NPs.

When NP diameter reaches 1 nm, it also becomes comparable to or smaller than another characteristic distance—the Bjerrum length (l_B), which describes the separation at which the energy of electrostatic interactions between ions is equal to the thermal energy in the media, $k_B T$ (k_B , Boltzmann constant; T , temperature). The PB and similar mean-field formalisms can be applied only when the charges are separated by distances much greater than l_B . This concept does not hold for the majority of interacting NPs (1–7, 11, 13, 14, 33, 34) because $l_B = 0.7$ nm for aqueous media and 28 nm

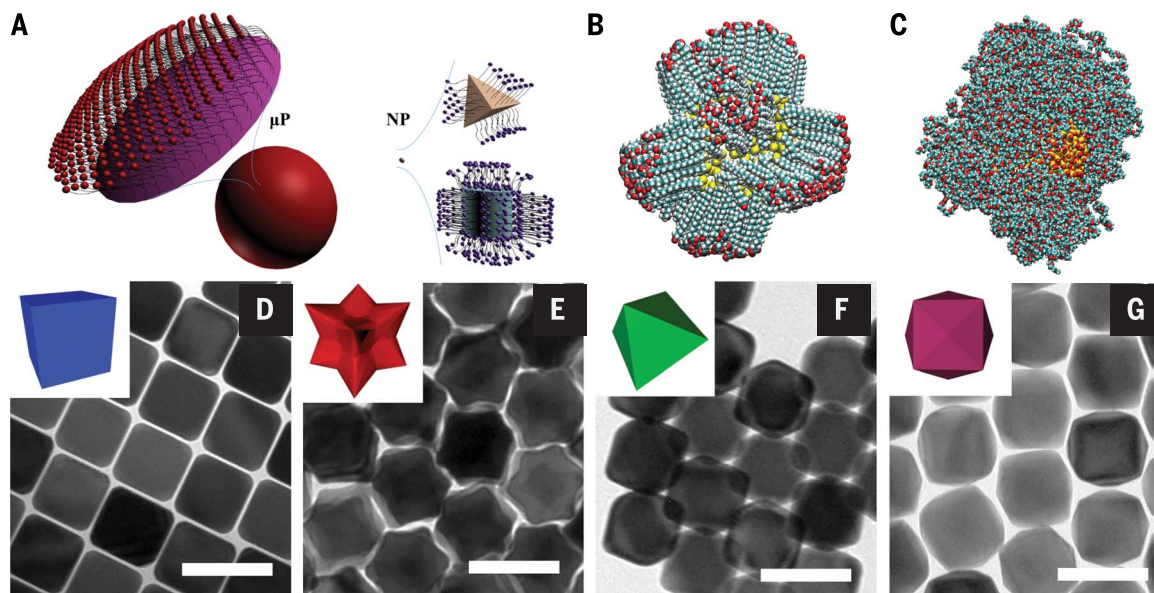


Fig. 1. Sizes and shapes of NPs. (A) Comparative pictorial representation of microscale and nanoscale particles. (B and C) MD simulations of 4-nm Au NPs coated with S-(CH₂)₁₇COOH (B) (23) and a 5-nm silica NP coated with (PEO)₁₀₀ (C) (22). Yellow, sulfur; cyan, carbon; red, oxygen; blue, nitrogen; white, hydrogen. (D to G) Transmission electron microscopy (TEM) images of Au NPs with the shapes of cubes (D), concave rhombic dodecahedra (E), octahedra (F), and tetrahexahedra (G) obtained by seed-mediated oxidative-reductive growth cycles (20). Scale bars in (D) to (G), 100 nm.

for heptane. When the distances between charges become smaller than l_b , multiple ion-correlation effects (see below) occur, and this contradicts the central assumption of the Poisson distribution in the foundation of this theory.

One approach to adapting PB formalism for NPs would be to consider surface potential (ψ_0) as a variable dependent on r and other system parameters. However, for NPs, an adequate expression for $\psi_0(r, d, I, \dots)$ must account for the dynamic surface layer and therefore will require addressing the same issue of structural discreteness of the NP-solvent interface. In addition, surface layers often display variations in surface grafting density.

Even if the grafting density were completely uniform, the interaction potential between NPs would be nonspherically symmetric (Fig. 1, B and C), unlike the ordinary DLVO potential. The asymmetry of the potential is greatly enhanced when NPs are faceted or truncated (35, 36).

An additional conceptual problem is that, when r and d are held constant, electrostatic and other potentials between the NPs fluctuate over time. Drastic fluctuations of V_{el} can be caused, for example, by single hydrated ions entering the interparticle space. These deviations cannot be described by typical colloidal-type potentials or steric repulsion (37, 38).

Illustration of deviations

Several experimental studies have demonstrated that the DLVO theory can provide an adequate description for particles of 50 nm and larger (39, 40).

For example, modeling of plasmonic interactions has shown that the average distance between citrate-stabilized 80-nm Au NPs appears to correlate well with DLVO predictions (41). However, as the particles become smaller, deviations occur. As long ago as 1971 (42), it was noted that the classical treatment of interparticle forces incorrectly predicts that nanoscale silica should coagulate (42, 43). Subsequent experimental (44–47) and computational (48) studies pointed out the unusual colloidal stability of NP dispersions under different media conditions. Contrary to the body of knowledge accumulated for μ Ps, a lack of a correlation was observed between the stability of NP dispersions and their charge or ionic strength—for instance, for 4-nm ZnO NPs (49) capped by humic acid or 2.3-nm Au NPs capped by mercaptoundecanoic acid (50). The deviations of the NP behavior were empirically rationalized by a specific packing of the interfacial layer, steric effects of the surface layer, preferential absorption of water at the NP interface, or incorporation of additional terms in Eq. 1, such as osmotic and elastic potentials (45, 51, 52). Thus, it is difficult to apply these principles to all NPs, and a different approach is needed.

Systematic behavioral deviations of NP dispersions are present across all material platforms. Even ligand-free Au NPs synthesized through laser ablation and stabilized by the tightly bound Stern layer of adsorbed ions were found to be stable against coagulation in the presence of F^- and SO_4^{2-} , whereas other anions with identical

electrostatic valence, such as I^- and SCN^- , destabilize these suspensions (47).

Looking beyond dispersions, unusual behavior due to nonclassical NP interactions was also observed for phase transitions. In particular, octadecanethiol-capped 1.7-nm Au NPs reversibly recrystallize from an amorphous solid to a body-centered cubic superlattice upon heating (Fig. 2A). This is in contrast to the classical thermodynamics (53) expectation that recrystallization would occur upon cooling (54).

Deviations from classical behavior can also be consistently seen in atomistic molecular dynamics (MD) simulations of NPs. For instance, the size of a counterion strongly affects particle interactions, contrary to the DLVO predictions (48, 50). As confirmed by experiments and simulations, a large counterion such as tetrabutylammonium hydroxide can provide a repulsive barrier, even at vanishing electrostatic potential (Fig. 2B). The presence of multiple extrema for 2-nm Au NPs revealed that the V_{el} displays a nonmonotonic dependence on interparticle center-to-center distance, contrary to predictions from PB formalism. Strong coupling of electrostatic, vdW, hydrophobic, and other forces accounts for this effect (Fig. 2C). Interparticle gaps $d < 2.5$ nm were found to be especially problematic, regardless of a specific NP diameter (48, 55, 56).

Substantial improvements in predictions of classical theories for ion concentration profiles may be achieved using classical liquid-state density functional theory (DFT) of the Carnahan-Starling type,

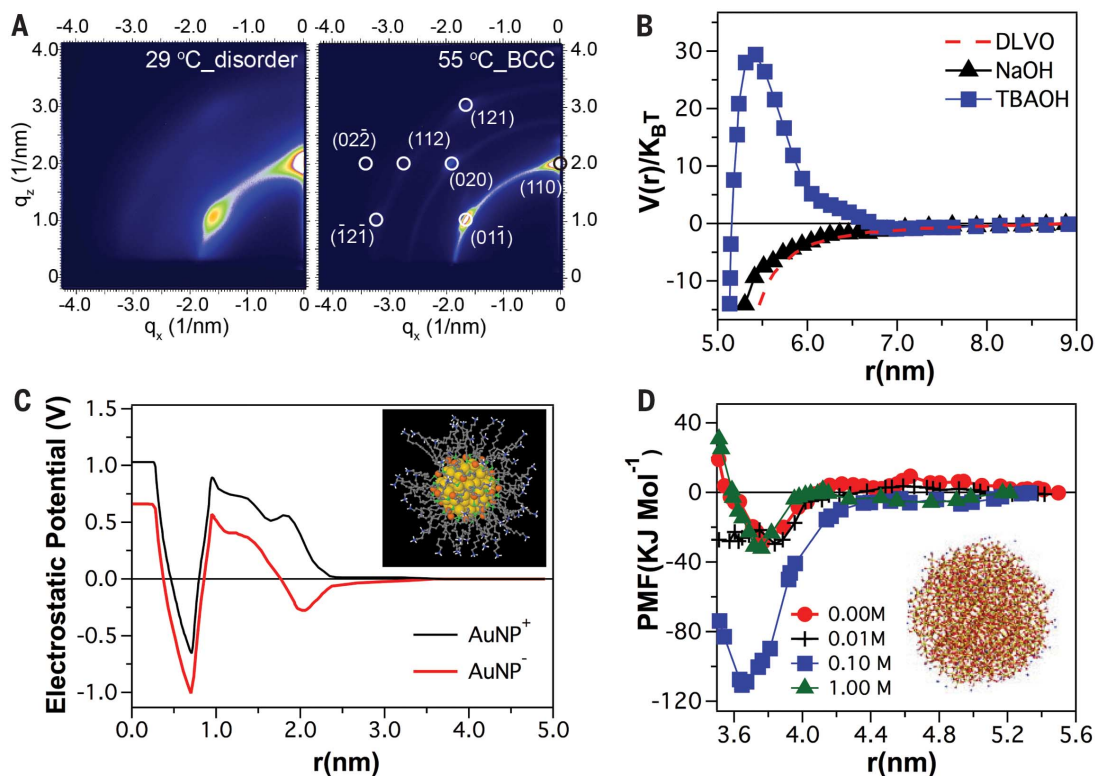
Fig. 2. Manifestation of nonadditivity of NP interactions in experiments and simulations.

(A) Grazing-incidence small-angle x-ray scattering patterns for octadecanethiol-capped 1.7-nm Au NPs undergoing reverse phase transition (53). NP solids transform from disordered to crystalline upon heating from 29° to 55°C.

(B) Comparison of the PMFs calculated according to DLVO (red) and MD simulations in the presence of positive ions of different diameters. Blue, tetrabutylammonium (TBA^+); black, Na^+ . [Redrawn from (48)]

(C) Radially integrated electrostatic potential in dispersions of 2 nm with different stabilizer layers: $Au_{144}(SR-NH_3^+)_{60}$ (black) and $Au_{144}(SR-COO^-)_{60}$ (red), where R is $-C_{11}H_{22}$. [Redrawn from (56)]

(D) PMF for a pair of two amorphous 4.4-nm SiO_2 NPs in the presence of $[Na^+] = 0.00, 0.01, 0.10, \text{ and } 1.00$ M. [Redrawn from (58)] Insets in (C) and (D) show atomistic images of NPs used in the simulations. Notice that abscissas in (B) and (D) refer to the center-to-center interparticle distance (r) for spheres of 5.1- and 3.2-nm diameter, respectively.



even when the medium is described as a dielectric continuum. DFT models are able to capture the oscillatory density profiles of small ions and the charge inversion phenomena for uniformly charged 1.5-nm particles (57). As another example, PMFs for a pair of 4.4-nm silica NPs are similar in shape to that expected from the DLVO theory. However, these PMFs displayed maximum attraction and the deepest potential well for intermediate electrolyte concentration (Fig. 2D) (58) or multiple PMF extrema (Fig. 2C) (56), whereas DLVO theory predicts a monotonic decrease of repulsive electrostatic interactions (and, thus, the total energy of particle interaction) as I increases. More generally, it was found that for anionic NPs, DLVO theory overestimates $V_{\text{el}}(r)$ and underestimates it for cationic NPs. Also, the location of the attractive well is strongly shifted toward longer distances (55), which correlates well with the enhanced colloidal stability observed experimentally.

Self-organization phenomena at the nanoscale reveal even more clearly the limits of the predictive power of classical theories when applied to NPs. Formation of closely packed NP films, assemblies (1–7, 33, 34, 59), and supraparticles (60) may be explained using hard-sphere, DLVO, or Yukawa-type PMFs. However, the differences between self-assembled structures found for μPs and NPs are particularly vivid because the close-range interactions make the errors of typical assumptions particularly influential. The entire spectrum of the crystal habits, including quasi-crystalline patterns, observed for NP superlattices cannot be explained by the classical PMFs (34). For instance, the inclusion of static dipolar interactions (i.e., Debye and Keesom forces) was found to be essential for explaining the packing of NPs into superlattices (33). The same is also true of NP assembly into chains, sheets, twisted ribbons, and shells (6, 7, 36, 61, 62), as well as for assemblies of NPs with other chemical species. When NPs are combined with proteins, multiple examples of discontinuities and counterintuitive trends are ascribed to “patchy” interactions (63–66). Many parallels in the behavior of NPs and globular proteins should also be noted (15, 67, 68). The strong influence of

even subtle PMF asymmetry can also be seen in the enantioselective self-organization of chiral NPs (62).

Nonadditivity at the nanoscale

New experimental techniques to study forces applied to macroscale surfaces (12), microscale particles, molecules, and ions have been instrumental for appreciation of the complexity of interfacial forces at nanometer-scale separations. Nonadditivity of all major classes of interactions becomes apparent when analyzing the diverse sets of data at both single-particle and ensemble levels. Nonadditivity ultimately originates from the discreteness of matter that becomes dominant when distances become smaller than several tens of nanometers. As one of the manifestations of the nonadditivity, PMFs with multiple extrema stemming from the interdependence of vdW, electrostatic, and hydrophobic interactions are observed (Fig. 2). The unusual stability of dispersions of small NPs (42–45), numerous NP assemblies with extraordinarily sophisticated geometries, biomimetic behavior of NPs in their complexes with enzymes (67, 69), and complex dynamics of protein coronas (70) represent experimental manifestations of nonadditivity.

The concept of interaction coupling is well known in molecular biology and is exemplified by ion-specific effects (ISEs) (16). More than a century ago, Franz Hofmeister recognized that ions with identical charge precipitate proteins to differing extents. It was later discovered that equally charged ions exhibit opposite tendencies to concentrate in the interfacial regions and can be classified by this tendency into chaotropes and kosmotropes. The Hofmeister series can be observed for many interfacial phenomena, usually when the Debye length is small. The order of ions in this series can be reversed when the polarity and/or the chemistry of the surface is changed.

Ion-specific effects stem from a complex set of nonadditive interactions between the ions, the solvent, and the surface; ISEs depend on the size, polarizability, and solvation of the ions, as well as on the hydrophobic or hydrophilic properties of the interface (71, 72). The origin of ISEs may be simpler

than it appeared at the time of Hofmeister. Due to discreteness of the matter at this scale, ions interact not only electrostatically but also via London dispersion interactions (73). At the first level of approximation and using the terms of the original theories, the potential of an ion within nanometers of the interface can be described as

$$V = V_{\text{el}} + V_{\text{ISE}}, \text{ where } V_{\text{ISE}} = V_{\text{i-vdW}} + V_{\text{solv}} + V_{\text{image}} \quad (2)$$

where V_{image} (74) and $V_{\text{i-vdW}}$ are image and van der Waals potentials, respectively, of ions near any surface, including that of NPs. As a simple demonstration of nonadditivity, $V_{\text{i-vdW}}$ is affected by the reorganization of the solvation shell and is therefore dependent on the solvation potential V_{solv} (75, 76). Nonadditivity of ionic interactions translates into nonadditivity of interparticle forces, because V_{ISE} modifies the counterion distribution in the vicinity of the NP interface (16) and thus affects V_{el} and the other contributions to the PMFs of NPs (71, 73). Although theoretical and simulation methods have been developed to describe ISEs (73, 77), accounting for them accurately requires the simultaneous inclusion of hydration, ionic size, and polarizability effects, defying many theories. These effects become even more convoluted and escape direct theoretical quantification based on Eq. 2, especially for NP surfaces that display a mixture of polar and nonpolar groups.

The complexity of nanoscale electrostatics also manifests in ion-ion correlation (IIC) effects that originate from the discreteness or finite sizes of solvated ions and their mutual interactions (78). The latter are ignored in the mean field approximation that eventually leads to incorrect predictions of V_{el} (79), V_{solv} , and V_{image} . IICs are implicated in the peculiar behavior of μPs that is often described as charge inversion (80), charge amplification (81), and attraction of like-charged particles (81, 82). Studies of these phenomena by atomic force microscopy (AFM) and optical tweezers have demonstrated that quantitative agreement with DLVO theory is possible for simple

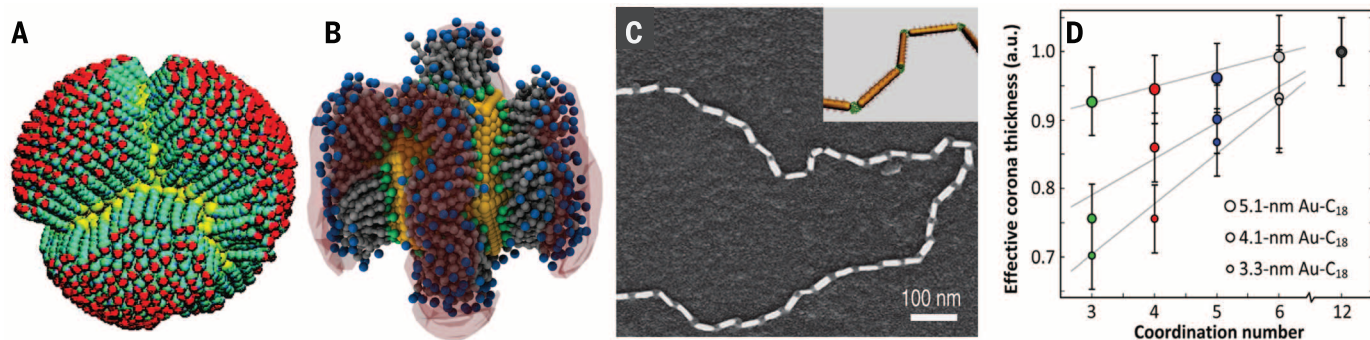


Fig. 3. Collective behavior of stabilizer molecules. (A and B) Snapshot of MD simulation of (A) spherical (65) and (B) icosahedral NPs (109) with charged groups at the core surface. For (B), isodensity surface of counterions and anions in the vicinity of the surface ligands is shown in pink. (C) Scanning electron microscopy image and schematic (inset) of the chains of end-modified gold nanorods (120). Scale bar, 100 nm. (D) Dependence of the effective thickness of the soft surface layer on the number of nearest neighbors in closely packed Au NPs (112). a.u., arbitrary units.

configurations of the individual μ Ps, but not for distances shorter than 5 nm (46), for multivalent ions (83), or for particles in the presence of additional surfaces (82), unless correction factors and additional interactions are incorporated.

The statistical mechanics of hard-sphere fluids has been an important theoretical tool in explaining these phenomena and has led to the development of several successful methods to describe IICs—namely, the liquid-state hypernetted chain (HNC) approximation, the Percus-Yevick approximation, and the mean spherical approximation. While providing a conceptual explanation for IIC phenomena, it was found that the effects of ionic size and Coulombic interactions are interdependent and that computational results defy expectations based on additivity of constitutive potentials (84, 85).

Ion-ion correlations are relevant within several ionic diameters (85) and a length scale of $\sim 3.6\lambda_{GC}$, where λ_{GC} is the thickness of the Gouy-Chapman layer (86, 87) of counterions (~ 2 to 4 nm) (88). At distances much larger than λ_{GC} (i.e., 20 to 100 nm), the IICs are weak and the distribution of ions can be described fairly accurately by the PB equation, which is consistent with the AFM experiments using latex μ Ps (88) and the quantitative theoretical account of IIC using the Yvon-Born-Green hierarchy (89).

Besides correlated dynamics of ions, excluded volume effects arising from the finite volume of ions make a large contribution to IICs. Hence, the ion density distributions do not monotonically decrease with distance from the surface but rather become oscillatory, reflecting the dimensions and molecular identity of solvated ions (78). Such oscillations can extend for distances of up to $14.5l_B$ (87) or, by other estimates, 15 nm (85).

Ion-ion correlations are especially important for charged NPs for which electrostatic interac-

tions between ions become much larger than the thermal energy. Thus, the counterion cloud behaves as a strongly correlated liquid. In nanocolloids, IICs may lead to an apparent attractive nanoscale-range interaction that is comparable in strength to vdW interactions and that increases in strength with NP charge (87).

Alteration of the structure of water around interfaces and solutes gives rise to intermolecular interactions, commonly known as hydrophobic interactions. Their potential, V_{hph} , is nonadditive with V_{el} and V_{vdW} (17). Molecular disturbances, which are essential for understanding hydrophobic interactions, were observed around NPs for distances of up to 2 nm for a variety of solvents—e.g., water (90), propanol, and ethanol (91). Similar ranges exceeding 1 nm were observed in MD simulations for C_{60} buckminsterfullerene and 2-nm Au NPs (92). Terahertz spectroscopy that directly probes solvation dynamics revealed the width of the dynamical hydration layer around proteins to be 2 nm as well (93).

Historically, hydrophobic interaction has been attributed to the reduction in entropy of liquid water upon solubilization of nonpolar molecules due to the formation of a rigid icelike cage by the water molecules around the solutes. More recently, however, simulation (94) and Raman scattering experiments (95) have demonstrated that this mechanism is valid only for small solutes. A transition from a structured hydration shell to a “dry” disordered shell with lower water density takes place when the solute reaches a size of ~ 1 nm (94). The interface of this dry disordered shell is formed by water molecules having “dangling” -OH groups with broken hydrogen bonds (96), and enthalpy rather than entropy dominates the free energy of solvation. Thus, for large solutes, both hydrogen bonds and dispersion interactions between solutes and solvents

make contributions to the balance of solvation energies.

Because electrostatic interactions can alter the organization of water at the interfaces, hydrophobic interactions become intrinsically dependent on the charge state of the interface and on the presence of ions in its vicinity. Therefore, these interactions are intertwined with electrostatic forces (97). Moreover, charge nonuniformity on a scale comparable to that of hydrophobic forces increases the interdependence of V_{el} and V_{hph} . Dynamic charge variations can stem from the stochastic distribution of ions and electrons and from local chemistry.

Initially, the coupling of hydrophobic forces with other interactions was suspected from simulations of proteins with hydrophobic patches (98). Very recently, chemical AFM measurements revealed a dramatic change in the strength of hydrophobic interaction on co-immobilization of amine or guanidine residues. These residues are surrounded by a different water shell; protonation of amine groups doubles the strength of hydrophobic interactions, whereas guanidinium groups eliminate measurable hydrophobic interactions in all of the pH ranges investigated (17).

Atomic multibody polarization effects are the key reason for the nonadditivity of the London dispersion forces. Their nonadditivity reflects the fact that the polarizability of an atom is affected by the dynamic polarization state of adjacent atoms and includes collective components of polarizability (99). In other words, integration of dispersion interactions between pairs of atomic voxels, assuming their independence, introduces large errors when used to assess the attraction of particles of nanoscale dimensions. For small molecules or relatively large particles with dimensions of several tens of nanometers, the errors do not exceed 10 to 15% (100). Conversely, the additive calculations using the Derjaguin approximation were shown

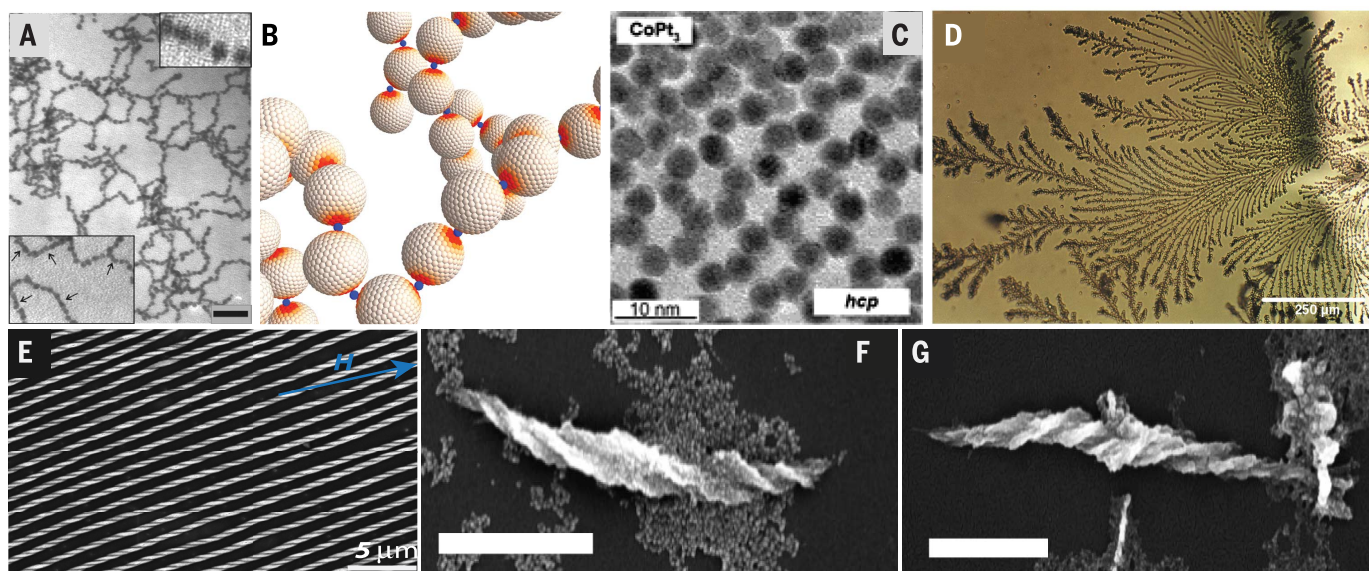


Fig. 4. Nonadditivity effects in self-organization of NPs. (A) Assembly of CdTe NPs in branched chains (6). Scale bar, 50 nm. (B) Simulated assemblies with multibody effects due to polarization (19). (C) One-layer superlattice from $CoPt_3$ NPs (33). (D) Dielectrophoretic assembly of dendrites from 15- to 30-nm Au NPs (127). (E) Assembly of ~ 10 -nm cubic magnetite NPs with helical packing as the result of the interplay between magnetic dipole coupling and close packing (128). (F and G) Enantioselective assemblies of chiral CdTe NPs into left- and right-winded helices (62). Scale bars in (F) and (G) are 150 nm.

to produce large errors for particles ~16 nm in diameter (101). Theoretical studies have shown that the interaction energy between small NPs asymptotes to the pairwise sum when the interparticle separation reaches about one particle diameter, and it deviates by 17% when the NPs are in near contact (102). However, in some cases the classical pairwise description is sufficiently accurate because the higher-order terms cancel each other (103). Such compensation occurs most often for isotropic bodies and is strongly dependent on geometry and separation distances between them. MD simulations for 1- to 5-nm silicon NPs (104) using the COMPASS force field yielded values of both attractive London dispersion and repulsive close-range Born forces that differed by several orders of magnitude from results based on their pairwise additivity.

The largest deviations are expected for highly polarizable (plasmonic) materials (105). The contribution of multibody effects doubles the attractive forces over those calculated by Hamaker theory for NPs in this case (102). The importance of nanoscale effects on dispersive interactions is further enhanced by size-dependent quantum confinement effects (106) and bond length variability (107) affecting the polarizability of atoms and particles.

A particularly vivid example of nonadditivity can be observed in the collective behavior of stabilizer ligands on a NP surface, representing multibody phenomena at molecular scale in NPs (Fig. 3). The collective behavior of surface ligands is the direct consequence of the multiplicity of interdependent molecular processes: grafting layer transitions, stabilizer entropy, faceting, solvent structuring, hydrogen bonding, hydrophobic interactions, and electrostatic repulsion of the charged groups at the solvent-ligand interface. In such a system, a change in the electrostatic component does not occur separately from the other interactions. Therefore, no sum of individually defined potentials can describe the interactions of such particles at distances comparable to NP diameters and ligand lengths, such as interparticle gaps of a few nanometers.

Spontaneous bunching of ligands is an illustration of collective behavior in surface layers of NPs (Fig. 3A) (65). Surface phase separation patterns can appear for chemically distinct stabilizers, as well as for surface ligands of different lengths that maximize the entropy of their head groups by surface segregation (108). The oligomeric character of the ligands and the solvent facilitates such behavior (35, 37, 102); faceting of the NPs can further enhance it as well (Fig. 3B) (35, 109), resulting in a 100× increase in both association constants and rates of NP aggregation (110).

The dynamic restructuring of the surface layer on NPs in close contact results in a considerable attractive force due to both entropic (111, 112) and enthalpic effects. The former are related to the tendency of the ligands to maximize their degrees of freedom. Besides the variety of intermolecular interactions mentioned above, the latter ones can also originate from fluctuations of the ligands (38), analogous to the London dispersion force. Based on MD simulations, the London-like interactions

from dynamic reconfiguration of the surface layer may contribute as much as $6k_B T$ to the total interaction potential (113). Interparticle forces at the nanoscale can, in fact, be dominated by collective ligand alignment that organizes nearby solvent molecules (114).

Together, the multibody effects in surface layers lead to Dzugotov-like (115) and more complex potentials (Fig. 2C) that, in turn, result in notable macroscopic effects exemplified by unexpected rotatory optical activity of dispersions (21), reverse temperature transition (Fig. 2A) (53), and formation of superlattices with complex periodicities (116) such as quasi-crystals (117, 118).

Nonspherically symmetric interactions owing to uneven ligand densities also play a role (119). Collective restructuring of the oligomers coating NPs maximizing hydrophobic interactions is implicated in the formation of chains of end-modified gold nanorods (120) and hexagonally packed mesoscale capsules (Fig. 3C) (121). Reconstruction and compressibility of the stabilizer layer on particle-particle contact strongly affect the effective NP diameter (Fig. 3D). This reconstruction typically increases the “stickiness” of NPs, promoting formation of amorphous solids (122, 123).

Multibody effects of NPs manifest themselves particularly vividly in large ensembles of interacting particles (124). Entropic effects can make an essential contribution for such NP systems. In a simple case of hard particles with PMFs containing only short-range repulsion and no attraction, the collective maximization of degrees of freedom of faceted particles similar to those in Fig. 1 results in their ordering into a variety of crystalline and quasi-crystalline superlattices (125). For more complex PMFs corresponding to dispersions, the rotational, translational, or vibrational motions of NPs become coupled with the entropy of solvent molecules, ions, and stabilizer molecules.

Collective behavior of NPs also reflects orientational preferences of NP association and angular anisotropy of PMFs. Anisotropy of interactions between individual NPs manifests particularly well in large NP ensembles; can be driven by internal or external electrostatic or magnetic fields; and can lead to NP self-organization into chains (Fig. 4, A and B), superlattices (Fig. 4C), or dendrites (Fig. 4D) (126, 127) and potentially many other extended assemblies. The collective behavior of NPs amplifies the effect of seemingly small energetic contributions to PMFs. For instance, static and dynamic dipolar polarization in the ground state of NPs is generally neglected but is capable of guiding the association into several common assembly patterns (6, 7, 19, 61). Evidence that weak interactions are capable of having large effects on the geometry of NP assemblies can also be observed in the formation of helical superstructures (Fig. 4, E to G) (61, 62, 128) from chiral (62) and nonchiral (128) NPs.

Heuristic rules

Heuristic rules can guide qualitative thinking about NP interactions, whereas quantitative understanding will require new theoretical and computational approaches, which we discuss next. Heuristic rules

could be helpful for discriminating between the systems where PB, DLVO, and other mean-field theories can and cannot be applied to NP interactions. Although this study indicates that universal laws regarding NP interactions are currently hard to come by due to diversity of mechanisms and scales of nonadditivity, the following trends emerge:

1) The use of PB theory for point charges is only reasonable for ion diameters that are less than 10% of NP diameter. For ions larger than this, liquid-state theory, involving the HNC or other approximations, becomes more useful.

2) Small ligands such as citrate ions might be considered simple stabilizers that do not respond to neighboring particles; when the stabilizer length becomes comparable to the NP diameter, the surface layer responds strongly to neighboring particles, producing large nonadditive interactions.

3) Solvent molecules with a characteristic length scale of 1 nm enhance nonadditivity of NP interactions (17, 94, 95).

4) Dynamic correlations between charged NPs become important when the electrostatic energy between them becomes comparable to $k_B T$.

5) The empirical Hofmeister series can provide guidance in predicting ion-specific effects in NP interactions (15). These expectations are confirmed by recent data (47, 129, 130).

6) Higher polarizability of the NP core must lead to stronger nonadditivity effects. This rule originates from the increase of the reconfigurability scale for electron density in NPs in response to external stimuli. It can be compared to the enhancement of non-DLVO behavior of polarizable ions (72). This rule manifested in theoretical studies of vdW interactions for NPs (103, 131).

These practical rules of thumb should help facilitate further research into NP interactions and aid molecular design of NPs for applications. In perspective, the multiplicity of nonadditivity mechanisms indicates that further conceptualization and theoretical development of this field must involve a new way of thinking about NPs as strongly correlated reconfigurable systems with diverse physical elements and multiscale coupling processes.

Atomistic potential of mean force for NP interactions

Besides highlighting the distinct issues and general regularities associated with NP interactions, we also want to begin answering the question of how to adequately treat the complex mutually correlated forces between NPs. Because of nonadditivity, all interactions must be considered simultaneously. Because of discreteness of matter at a scale relevant to NP interactions, molecular- and atomic-scale phenomena cannot be averaged out. In other words, a reasonable correlation with experiment cannot be expected by adding smooth potentials for electrostatic, dispersive, hydrophobic, magnetic, and other interactions individually corrected for nanoscale effects. Fortunately, molecular simulation methods, which can address both discreteness and nonadditivity, have reached impressive levels of speed and reliability.

The reduced dimensions of the nanoscale and the corresponding reduction in numbers of atomic degrees of freedom become a distinct advantage when simulating NPs with atomistic resolution. Also, advances in electron microscopy afford accurate visualization of the three-dimensional (3D) geometry of NPs, allowing the possibility of direct validation of molecular simulations. In fact, we are now at a juncture at which the accuracy of synthesis, the length and time scales of molecular simulation, and the imaging capabilities all overlap at the “sweet spot” of NP dimensions.

Thus, a rational strategy for future progress in the understanding of NP interactions is to use these capabilities to bypass enumeration of individual forces and to determine the net interparticle PMF directly from atomistic simulations. This strategy enables adequate accounting for nonadditivity of interactions at the nanoscale, exemplified by the difficulties of incorporation of ISE, IIC, and hydrophobic potentials as independent terms in, for instance, Eq. 1. Hydrogen bonding, capillary action, and other forces that may contribute to self-organization of NPs (1, 11, 13, 62) can be included with this method as well. Simulated PMFs can be verified by predicting the stability of nanocolloids and NP self-assembly patterns. In some cases of larger particles, additional verification can be obtained by taking advantage of liquid-cell electron microscopy techniques (Fig. 5, A and B) and extracting the PMF directly from NP diffusion (132).

Although the calculation of PMFs directly from molecular simulation remains in its infancy, recent work indicates that this strategy is viable. An example is the MD simulation of the PMFs of CdS nanorods stabilized by octadecyl thiol in *n*-hexane (Fig. 5, C and D) (114). Unlike the Hamaker theory, the simulations capture the complex interplay of the dispersion forces between the inorganic rods, the collective behavior of surface ligands, and the ordering of solvent molecules, providing a detailed picture of interactions between the nanoscale particles (133). The progressive changes in physical properties with varying particle sizes (1 to 4 nm) of metal (133, 134) and semiconductor NPs (135) containing as many as 8217 atoms can also be realistically described by the MD technique.

Despite shared force-field problems with biomolecules, NPs present a favorable system for atomistic simulations as compared with proteins and polymers. Solid inorganic cores have many fewer degrees of freedom than flexible organic chains, making NPs more realistic for MD, Monte Carlo (MC), and other simulation algorithms with limited computer power.

Considering the emerging possibility of an MD-PMF strategy for nanoscale systems, several important challenges must be mentioned. One challenge is the lack of sufficient computer power to run large simulations with atomistic resolution. This limitation is particularly notable when one needs to incorporate quantum calculations with MD code. These calculations are needed to

adequately describe interactions of atoms in the NP core and surface ligands.

Although the computer power limitation fades with each passing month, there are several other caveats. MD simulations have been used extensively in structural biology to resolve similar difficulties originating from the nonadditivity of nanoscale interactions and dynamics of proteins, DNA chains, lipid membranes, etc. Their success, however, has been limited because the existing MD force fields inaccurately describe many intermolecular interactions, especially hydrogen bonds. Second, the difficulties with including entropic contributions of the chemical groups and hydrophobic interactions should also be noted. Third, dispersion interactions between atoms are usually described in MD codes by pairwise summation of atomic Lennard-Jones potentials that ignore the polarization multibody effects. Last, the pairwise PMF neglects higher-order multibody effects that arise from influences of other particles on the particle pair and vice versa. So, the development of a PMF via simulation, while accounting for the nonadditivity of electrostatic, dispersive, and other forces between two NPs, proceeds by assuming additivity of interactions at the atomic scale and still leaves the assumption of nonadditive interactions among multiple NPs. Thus, traditional atomistic simulations are not a panacea but must be augmented by improved force fields and additional computational tools to address remaining issues of nonadditivity.

The existing MD force fields were developed and optimized for organic and biological molecules. They are suitable to describe the collective behavior of stabilizer ligands on a NP surface. However, force fields optimized for inorganic NPs are a rarity (135). Going forward, improvements in MD force fields need to be made while accounting for the specifics of NP interactions. As such, they should include a more adequate account of the nonadditivity of dispersion interactions. The Dzyaloshinskii-Lifshitz-Pitaevskii (DLP) theory (136, 137) and the coupled-dipole method (CDM) (99, 102) are currently the most promising approaches for evaluating dispersion forces. Nevertheless, both DLP theory and CDM are rarely used outside of the realm of theoretical physics, with a few exceptions involving μ Ps or NPs as noted below.

Continuum DLP theory (28) accounts for multibody effects, complex electrodynamics of atomic ensembles in the presence of an intervening medium, and retardation of electromagnetic waves through the use of dielectric function determined either experimentally (138) or computationally (139). The application of DLP to NPs has been limited, but a few studies demonstrate a strong mismatch between dispersion forces calculated with and without polarization multibody effects, especially for organic solvents (140) and small particles of few-nanometer size (141). The limitation of DLP theory is that the molecular and atomic details of the particle's interface are smeared out, which inhibits its ability to account for facets, apexes, or edges. This theory will also have

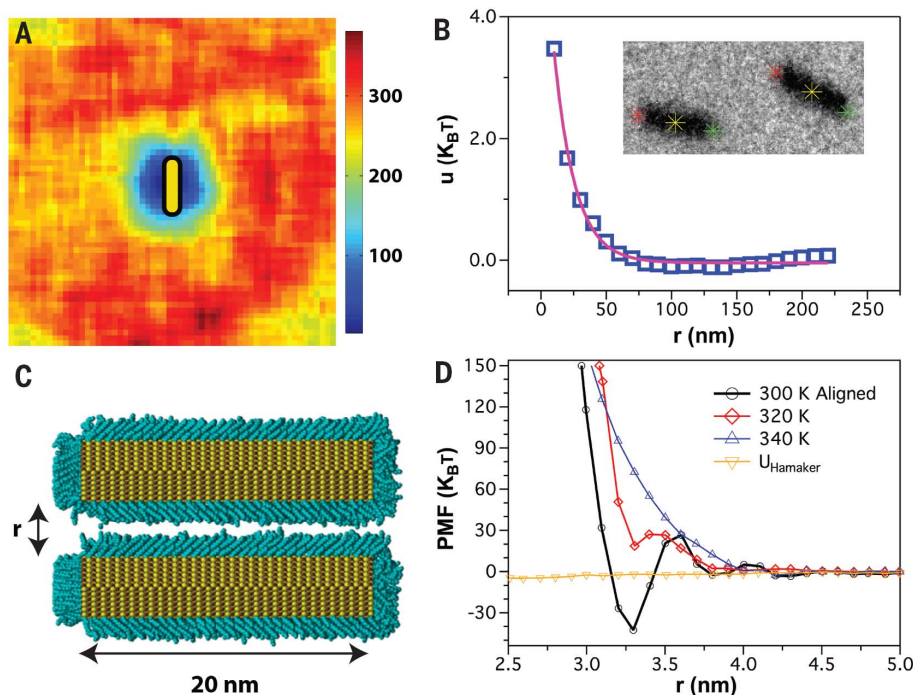


Fig. 5. Experimental and computational methods to obtain potential of mean force. (A) Color-coded counts of the total number of rods in the 2D plane pixels obtained by the real-time monitoring of nanorod behavior in liquid by TEM (132). (B) Experimental PMF for Au nanorods (inset) obtained by TEM imaging in liquid cells. (C) CdS 4-nm-by-20-nm faceted nanorods coated with alkyl thiol molecules and (D) their simulated PMFs at the temperatures indicated in *n*-hexane, where r is the distance between the opposing crystal facets (114).

difficulties accounting for NP surface dynamics that is essential at close separations.

The coupled-dipole method represents a fluctuation-based approach, similar to DLP, but remains valid when a continuum description of the NPs is not applicable. CDM views atoms individually as simple harmonic oscillators. Simultaneously, coupling of oscillators (i.e., atomistic multibody interactions) is allowed, thus avoiding atomic-level pairwise additivity. Because finding eigenvalues (frequencies) of this large system of coupled atomic oscillators is essential for CDM, its size represents one of the limiting factors for this approach, which we believe can be successfully resolved, even with current codes and computational hardware. The polarizability of atoms is usually described by the Drude model, but it often results in the so-called “polarizability catastrophe” for closely spaced atoms. Future improvements of CDM should include *ab initio* methods to calculate atomic polarizability, such as time-dependent DFT (142, 143), that can also incorporate retardation effects. Integration of quantum mechanics with CDM would make it possible to discriminate the electronic characteristics of atoms located in different parts of NPs (144). Validation of the different options for quantum mechanics-enhanced CDM models can be made using the well-established properties of individual NPs.

It also will be important to develop MD force fields that account for quantum confinement effects, as well as the diversity and specificity of atomic arrangements in NPs. Although there are numerous examples of successfully using quantum mechanics to calculate atomic charges, such methods are applicable only to relatively small atomic systems. Similar issues arise in any attempt to integrate CDM-based algorithms into MD simulations. In particular, the dynamics of the solvent and ions around NPs will require recalculation of parameters for dipolar coupling in every MD step due to time-varying interatomic distances. The computational costs for such a method could perhaps be reduced by using CDM on small subsystems to tune or “train” MD parameter sets specifically for each system, before a regular MD simulation.

Because computer speed is constantly increasing, we shall arrive at fairly accurate calculations of pairwise PMFs of NPs at some point in the future. The effects of NP material, size, ligand coating, and ions on NP interactions can then be systematically studied and, if necessary, deconvoluted. Then, pairwise additivity at the level of NP-NP interactions will need to be tested and multibody corrections made, which will be particularly essential for understanding the self-assembly phenomena. Testing the results of such simulations is increasingly made possible by experimental advances such as liquid-cell electron microscopy. Large ensembles of NPs would be best described by transitioning to coarse-grained models (125, 145). Besides MD, MC, dissipative particle dynamics, and other algorithms could be used at this point, depending on the scientific question. Some of them have already been used for μ Ps and NPs after PMFs were determined.

Coarse-grained models have demonstrated an ability to describe systems containing many thousands of particles, which is large enough to reach micrometer-scale dimensions. Simulations at this scale can be tested by many other experimental methods, including traditional optical microscopy, to verify coarse-grained models and observe temporal transformations. Thus, despite the challenges, there is reason for optimism that exciting progress in understanding, predicting, and exploiting the distinct properties of NP assemblies is on the horizon.

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RESEARCH ARTICLE SUMMARY

MARTIAN GEOLOGY

Deposition, exhumation, and paleoclimate of an ancient lake deposit, Gale crater, Mars

J. P. Grotzinger,* S. Gupta, M. C. Malin, D. M. Rubin, J. Schieber, K. Siebach, D. Y. Sumner, K. M. Stack, A. R. Vasavada, R. E. Arvidson, F. Calef III, L. Edgar, W. F. Fischer, J. A. Grant, J. Griffes, L. C. Kah, M. P. Lamb, K. W. Lewis, N. Mangold, M. E. Minitti, M. Palucis, M. Rice, R. M. E. Williams, R. A. Yingst, D. Blake, D. Blaney, P. Conrad, J. Crisp, W. E. Dietrich, G. Dromart, K. S. Edgett, R. C. Ewing, R. Gellert, J. A. Hurowitz, G. Kocurek, P. Mahaffy, M. J. McBride, S. M. McLennan, M. Mischna, D. Ming, R. Milliken, H. Newsom, D. Oehler, T. J. Parker, D. Vaniman, R. C. Wiens, S. A. Wilson

INTRODUCTION: Remote observational data suggest that large bodies of standing water existed on the surface of Mars in its early history. This would have required a much wetter climate than that of the present, implying greater availability of water on a global basis and enhanced potential for global habitability. However, based on assumptions of a vast water inventory and models of atmospheric erosion, theoretical studies suggest a climate that was wetter but not by enough to sustain large lakes, even in depressions such as impact craters.

RATIONALE: The Mars Science Laboratory mission's rover, Curiosity, provides the capability to test hypotheses about Mars's past climate. The focus of the mission is the exploration of a ~5-km-high mountain, Aeolis Mons (informally known as Mount Sharp), located near the center of the ~140-km-wide Gale impact crater. Mount Sharp is underlain by hundreds of meters of sedimentary rock strata deposited ~3.6 billion to 3.2 billion years ago. These sediments accumulated in aqueous environments, recording the history of Mars's ancient climate. Because of Curiosity's ability to study these strata where they are exposed near the base of Mount Sharp, we can directly test the hypothesis that large impact craters were capable of accumulating

and storing water as lakes for substantial periods of time.

RESULTS: Over the course of 2 years, Curiosity studied dozens of outcrops distributed along a ~9-km transect that also rose ~75 m in elevation. Image data were used to measure the geometry and grain sizes of strata and to survey the textures associated

with sediment deposition and diagenesis. Erosion of Gale's northern crater wall and rim generated gravel and sand that were transported southward in shallow streams. Over time, these stream deposits advanced toward the crater interior, transitioning downstream into finer-grained (sand-sized), southward-advancing delta deposits. These deltas marked the boundary of an ancient lake where the finest (mud-sized) sediments accumulated, infilling both the crater and its internal lake basin. After

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infilling of the crater, the sedimentary deposits in Gale crater were exhumed, probably by wind-driven erosion, creating Mount Sharp. The ancient stream and lake deposits are erosional remnants of superimposed depositional sequences that once extended at least 75 m, and perhaps several hundreds of meters, above the current elevation of the crater floor. Although the modern landscape dips northward away from Mount Sharp, the ancient sedimentary deposits were laid down along a profile that projected southward beneath Mount Sharp and indicate that a basin once existed where today there is a mountain.

CONCLUSION: Our observations suggest that individual lakes were stable on the ancient surface of Mars for 100 to 10,000 years, a minimum duration when each lake was stable both thermally (as liquid water) and in terms of mass balance (with inputs effectively matching evaporation and loss of water to colder regions). We estimate that the stratigraphy traversed thus far by Curiosity would have required 10,000 to 10,000,000 years to accumulate, and even longer if overlying strata are included. Though individual lakes may have come and gone, they were probably linked in time through a common groundwater table. Over the long term, this water table must have risen at least tens of meters to enable accumulation of the delta and lake deposits observed by Curiosity in Gale crater. ■



Inclined strata in the foreground dip southward toward Mount Sharp and represent ancient delta deposits. These deposits transition into strata in the mid-field that were deposited in ancient lakes. The buttes and mesas in the background contain younger deposits that overlie and postdate the lake deposits beneath Mount Sharp. The outcrop in the foreground is about 6 m wide, and the buttes and mesas in the background are hundreds of meters wide and tens of meters high. The image has been white-balanced. [Credit: NASA/Caltech/JPL/MSSS]

The list of author affiliations is available in the full article online.

*Corresponding author. E-mail:

grotz@gps.caltech.edu

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RESEARCH ARTICLE

MARTIAN GEOLOGY

Deposition, exhumation, and paleoclimate of an ancient lake deposit, Gale crater, Mars

J. P. Grotzinger,^{1*} S. Gupta,² M. C. Malin,³ D. M. Rubin,⁴ J. Schieber,⁵ K. Siebach,¹ D. Y. Sumner,⁶ K. M. Stack,⁷ A. R. Vasavada,⁷ R. E. Arvidson,⁸ F. Calef III,⁷ L. Edgar,⁹ W. F. Fischer,¹ J. A. Grant,¹⁰ J. Griffes,¹ L. C. Kah,¹¹ M. P. Lamb,¹ K. W. Lewis,¹² N. Mangold,¹³ M. E. Minitti,¹⁴ M. Palucis,¹ M. Rice,¹⁵ R. M. E. Williams,¹⁴ R. A. Yingst,¹⁴ D. Blake,¹⁶ D. Blaney,⁷ P. Conrad,¹⁷ J. Crisp,⁷ W. E. Dietrich,¹⁸ G. Dromart,¹⁹ K. S. Edgett,³ R. C. Ewing,²⁰ R. Gellert,²¹ J. A. Hurowitz,²² G. Kocurek,²³ P. Mahaffy,¹⁷ M. J. McBride,³ S. M. McLennan,²² M. Mischna,⁷ D. Ming,²⁴ R. Milliken,²⁵ H. Newsom,²⁶ D. Oehler,²⁷ T. J. Parker,⁷ D. Vaniman,¹⁴ R. C. Wiens,²⁸ S. A. Wilson¹⁰

The landforms of northern Gale crater on Mars expose thick sequences of sedimentary rocks. Based on images obtained by the Curiosity rover, we interpret these outcrops as evidence for past fluvial, deltaic, and lacustrine environments. Degradation of the crater wall and rim probably supplied these sediments, which advanced inward from the wall, infilling both the crater and an internal lake basin to a thickness of at least 75 meters. This intracrater lake system probably existed intermittently for thousands to millions of years, implying a relatively wet climate that supplied moisture to the crater rim and transported sediment via streams into the lake basin. The deposits in Gale crater were then exhumed, probably by wind-driven erosion, creating Aeolis Mons (Mount Sharp).

Substantial bodies of standing water may have existed on the surface of Mars in its early history. Large craters (1), the Valles Marineris canyon network (2), and even the hemispheric-scale northern plains (3) are depressions where water may have pooled. To support such widespread distribution of surface water, the planet's past climate must have been much wetter than it is today, enhancing the potential for past global habitability. Theoretical studies of Mars's ancient climate, based on assumptions of water inventory and using models of atmospheric loss, have suggested a climate that was wetter than at present but not by enough to sustain perennial lakes in topographic depressions (4).

The Mars Science Laboratory (MSL) mission to Gale crater, using the Curiosity rover, provides the opportunity to directly test hypotheses regarding Mars's past climate. Having discovered evidence for ancient stream and thin (meters-thick) lake deposits within its landing region (5), Curiosity recently explored the basal strata of Gale crater's central mountain, Aeolis Mons (informally known as Mount Sharp). Rocks that compose the foundation of this mountain include hundreds of meters of strata of unknown origin that contain evidence of hydrated minerals. We documented the succession of sedimentary rock types in this region and interpreted them in terms of past depositional processes, thereby enabling reconstructions of ancient environments and paleoclimate. In addition, we used measure-

ments of the thickness of stratified rocks on the surface today, combined with calculations of the likely extent of crater-wall backwasting and rim erosion, to perform mass-balance calculations and infer the processes by which Mount Sharp formed.

Geologic setting

Gale crater is located on fluvially dissected highland crust along the martian topographic dichotomy boundary. Crater counts on Gale's ejecta blanket and evidence from the underlying highland crust suggest that the crater formed ~3.8 billion to 3.6 billion years ago (6, 7). The interior plains of northern Gale crater (Aeolis Palus) contain remnants of eroded alluvial fan deposits and a large sedimentary mound (Mount Sharp) with the crater's central peak at its core, which may be exposed on the south side of the mound (5–9). Crater counts for Aeolis Palus suggest deposition of sedimentary materials before 3.3 billion to 3.1 billion years ago (fig. S1).

Curiosity explored two stratigraphic groups of sedimentary rocks exposed in Aeolis Palus and at the base of Mount Sharp (Figs. 1 and 2). We designate the Bradbury group as the rocks within Curiosity's landing ellipse, as well as those to the south of the ellipse, up to the contact with the Murray formation (Fig. 1). The Mount Sharp group comprises the rocks of Mount Sharp that show evidence from orbit of hydration (10); these include the basal Murray formation and the overlying hematite-, clay-, and sulfate-bearing

units (10, 11), up to an unconformity (8), which appears to separate rocks bearing hydrated minerals below from rocks without obvious hydrated minerals above (Fig. 1). Rover observations reveal that the mean dip of the Bradbury group is approximately horizontal, which allows elevation to be used as a surrogate for stratigraphic position in areas of poor bedrock exposure. Strata of the Bradbury group (Fig. 2) were studied along Curiosity's traverse (supplementary text) from Bradbury Landing, to the Yellowknife Bay study site, and then to the Pahrump Hills area, where the boundary between the Bradbury and Mount Sharp groups is exposed (Fig. 1). These rocks are described in ascending order.

Bradbury group sedimentology

Rocks of the Yellowknife Bay formation are composed dominantly of lacustrine mudstone and fluvial sandstone, with minor eolian sandstone (5, 12). Patchy bedrock exposures between Yellowknife Bay and Kimberley become more extensive to the south of Dingo Gap (Fig. 1 shows the locations referenced in this paper), where the rover entered a set of shallow valleys with exposed rock walls and floors. These well-exposed outcrops exhibit several distinct sedimentary facies (Fig. 2).

¹Division of Geologic and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA.

²Department of Earth Science and Engineering, Imperial College London, London SW7 2AZ, UK. ³Malin Space Science Systems, Post Office Box 910148, San Diego, CA 92121, USA.

⁴Department of Earth and Planetary Sciences, University of California–Santa Cruz, Santa Cruz, CA 95064, USA.

⁵Department of Geological Sciences, Indiana University, Bloomington, IN 47405, USA. ⁶Department of Earth and Planetary Sciences, University of California–Davis, Davis, CA 95616, USA. ⁷Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109, USA. ⁸Department of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, MO 63130, USA. ⁹Astrogeology Science Center, U.S. Geological Survey, Flagstaff, AZ 86001, USA.

¹⁰Center for Earth and Planetary Studies, National Air and Space Museum, Smithsonian Institution, Washington, DC 20560, USA. ¹¹Department of Earth and Planetary Sciences, University of Tennessee, Knoxville, TN 37996, USA.

¹²Department of Earth and Planetary Sciences, Johns Hopkins University, Baltimore, MD 21218, USA. ¹³Laboratoire Planétologie et Géodynamique de Nantes–Le Centre National de la Recherche, Unité Mixte de Recherche 6112 and Université de Nantes, 44322 Nantes, France. ¹⁴Planetary Science Institute, Tucson, AZ 85719, USA. ¹⁵Department of Geology, Western Washington University, Bellingham, WA 98225, USA. ¹⁶Department of Space Sciences, NASA Ames Research Center, Moffett Field, CA 94035, USA. ¹⁷NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA.

¹⁸Department of Earth and Planetary Science, University of California–Berkeley, Berkeley, CA 94720, USA. ¹⁹Laboratoire de Géologie de Lyon, Université de Lyon, 69364 Lyon, France. ²⁰Department of Geology and Geophysics, Texas A&M University, College Station, TX 77843, USA. ²¹Department of Physics, University of Guelph, Guelph, Ontario N1G 2W1, Canada. ²²Department of Geosciences, Stony Brook University, Stony Brook, NY 11794-2100, USA. ²³Department of Geological Sciences, University of Texas at Austin, Austin, TX 78712, USA. ²⁴Astromaterials Research and Exploration Science Division, NASA Johnson Space Center, Houston, TX 77058, USA. ²⁵Department of Geological Sciences, Brown University, Providence, RI 02912, USA.

²⁶Institute of Meteoritics, University of New Mexico, Albuquerque, NM 87131 USA. ²⁷LZ Technology, NASA Johnson Space Center, Houston, TX 77058, USA. ²⁸Space Remote Sensing, Los Alamos National Laboratory, Los Alamos, NM 87544, USA.

*Corresponding author. E-mail: grotz@gps.caltech.edu

In order of decreasing frequency of exposure, these include crudely stratified to cross-stratified sandstone and pebbly sandstone, conglomerate, clinoform sandstone, and finely stratified sandstone.

The crudely stratified to cross-stratified sandstones consist of poorly sorted to moderately well sorted, fine- to coarse-grained sands, with decimeter-scale sets of trough crossbeds (fig. S2). Coarser-grained beds define the base of multiple upward-fining sequences. Poorly sorted pebble-rich sandstones also are common. Pebbles are dispersed throughout the sandstone framework, reach up to ~22 mm in diameter, and are sub-angular to subrounded in shape (12). The facies is

grain-supported but shows no preferred grain orientation. Elongate fine-grained clasts may be mudstone intraclasts (fig. S2).

The conglomerates are interstratified with sandstones and pebbly sandstones (12, 13) and are present throughout the Bradbury group. Characteristically, they are weakly stratified, poorly sorted, and composed of angular to subrounded clasts (fig. S2) that reach up to 6 cm in diameter. Beds of conglomerate are 10 to 30 cm thick.

The presence of grain-supported sandstones and pebbly sandstones and the common occurrence of cross-stratification provide strong evidence for bedload sediment transport in an ancient

fluvial system. We also interpret the conglomerates as evidence of deposition in a fluvial environment, formed by the migration of subaqueous bedforms or barforms, similar to conglomerates observed between Bradbury Landing and Yellowknife Bay (13). The low-to-moderate level of rounding is consistent with a short transport distance, such as deposition in an alluvial fan environment.

Finely stratified sandstones occur rarely in the Bradbury group and are characterized by regular millimeter-scale laminae that form thin sheets 5 to 10 cm thick and, rarely, dune-scale cross-stratification. In one case, the fine laminae that make up the foresets of a small dune are themselves cross-stratified (fig. S2), indicating centimeter-scale compound cross-stratification. The fine regular lamination is similar to “pinstripe” lamination, which is produced by the migration of eolian impact ripples (14).

Climoform sandstones

An unusual texture, characterized by distinct striations and here termed “orbital striated outcrop” (OSO), is observed in images collected by the High Resolution Imaging Science Experiment (HiRISE) orbiter (Fig. 3A). The OSO texture is exposed within dissected parts of southern Aeolis Palus (Fig. 3B) and is typified by striations consistently trending east by northeast-west by southwest (trend direction of N65°E) that extend up to ~100 m along strike within individual outcrops. The OSO is exposed discontinuously in topographic lows that reflect partial exhumation from beneath overlying strata (supplementary text). These exposures of OSO are distributed over an area ~3 km in the east-west direction and ~2.5 km in the north-south direction (Fig. 3B). Striations are expressed in HiRISE images as alternating bands with light-dark albedo variations (Fig. 3A).

Rover observations relate the OSO texture to sedimentary rocks with distinctive geometries, known as clinoform sandstones (Fig. 4). Clinoforms are inclined stratal surfaces, found within volumes of sediments and sedimentary rocks, that occur over a wide range of spatial scales and depositional settings (15). Typically, they comprise a low-gradient topset that transitions into a steeper-gradient foreset zone, which may transition into low-gradient bottomsets. They are distinct from crossbedding in that they are larger in scale, with planar to sigmoidal (rather than trough-shaped) foreset geometry, and have a consistent dip direction when measured over kilometer-length scales (16).

Rover observations of the OSO show clinoform geometries that dip 10° to 20° to the south. In addition, rover observations of valley walls reveal clinoform sandstones that cannot be observed from orbit. Observations of the upper and lower bounding surfaces of these inclined sets of strata show that the clinoforms range from 1 to 4 m in thickness.

Observations of the clinoform sandstones at the north Kimberley “Square Top” target show that they are composed of fine sands with dispersed coarser grains (Fig. 4C). The latter are subangular to rounded, coarse to very coarse grains, with

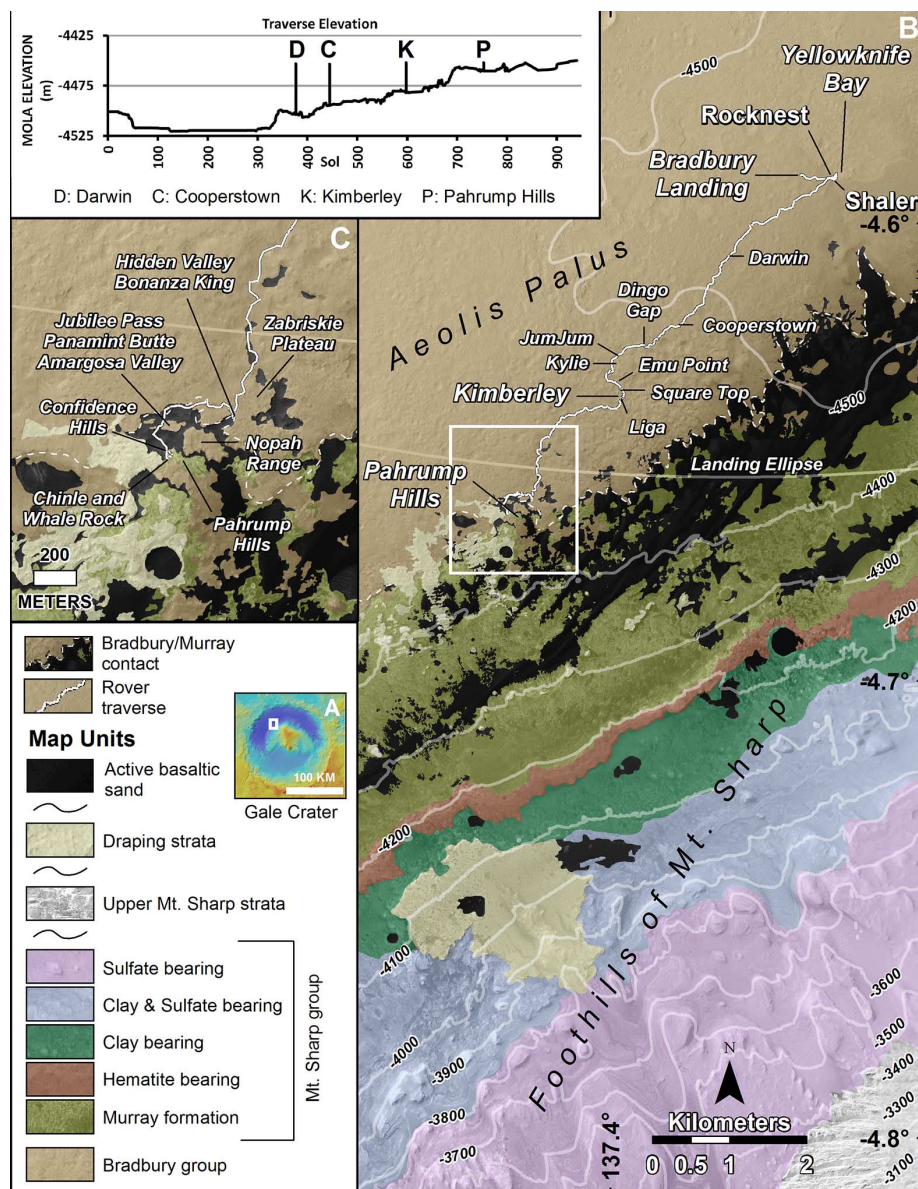


Fig. 1. Regional geologic map showing the location of the rover traverse and key study areas. Inset map (A) shows Gale crater topography; the white box outlines the area shown in (B). (B) Geologic map showing the principal stratigraphic units exposed at Aeolis Palus and the foothills of Mount Sharp, the major study locations, the rover traverse, and the landing ellipse. The white box outlines the area shown in (C). (C) Map details and locations of study points in the vicinity of Pahrump Hills. (Top inset) Topographic profile of the rover traverse.

the larger grains ranging from 0.5 to 3.1 mm in diameter and with an average grain diameter of 1.0 to 1.4 mm. An underlying conglomerate bed contains subangular to subrounded grains up to 3 mm in diameter. Farther south, inclined, interstratified granule- and pebble-rich beds and sandstone beds marked by poorly sorted, subangular to subrounded grains are present (Fig. 4D). The largest grains at the “Liga” target are 3.1 to 4.7 mm in diameter. Such coarse grain sizes indicate transport by water rather than by wind.

Recessive and probably poorly cemented dipping conglomerates are interstratified with resistant, well-cemented sandstones at Kylie and Kimberley (Fig. 4C). The repetition of beds with

different resistances to erosion may result from differential cementation of beds with different grain sizes. Beds that form clinoform foresets are massive to poorly stratified, suggesting emplacement on foreset surfaces via downslope grain flows.

Only a few complete exposures of clinoforms are observable, and these have sigmoidal geometries (17), in which gently dipping topset beds steepen and transition into south-dipping foreset beds that become shallow at their bases. A distinct transition from topset to foreset geometry is apparent at the north end of the Kimberley outcrop.

More generally, the foreset beds are truncated at their tops by erosional surfaces capped by subhorizontal strata (Fig. 4B). These truncating

strata have variable characteristics that probably reflect different formation processes. In places, these strata appear to be coarser-grained sandstones and conglomerates, which we interpret as fluvial topsets. Elsewhere, these truncating strata are fine-grained, thin-bedded sandstones with small-scale cross-stratification, probably also of fluvial origin. Locally, bedsets of small-scale cross-stratification with northward paleoflow indicators suggest eolian reworking of previously deposited fluvial sands.

Exposures of OSO rise in elevation from -4502 to -4460 m from north to south (Fig. 3C), which indicates that clinoform sandstones also should occur at stratigraphically higher levels to the south, toward Mount Sharp. When compared with the elevation of clinoform sandstones observed at Kylie, rover observations confirm that south-dipping clinoform sandstones occur ~10 m higher at Kimberley, ~25 m higher at Zabriskie Plateau and in Hidden Valley, and ~30 m higher in the southwest arm of Hidden Valley.

Synthesis: Reconstructing ancient deltas

The consistent strike of the OSO clinoform sandstones across hundreds of meters (in map view) and the presence of consistent south-dipping beds (observed in cliff-face sections) tightly constrain the mode of deposition for these sandstones. On Earth, inclined foresets of sand and gravel can be deposited by migrating river dunes, river bars, and delta mouth bars. In each case, inclined foresets are produced by sediment accretion at the margins of depositional bedforms. Fluvial barforms, such as scroll bars, are curved in the strike direction at scales of tens to hundreds of meters, because they reflect the growth of point bars in meandering river systems (18–20). Moreover, planform shapes of bars vary spatially from one bar to another (20, 21). Similarly, bars in braided rivers show complex patterns of sediment accretion, with bar growth and inclined strata development both downstream and at the lateral margins of bars (22–24). The absence of marked curvature in the OSO striations, both within outcrops and across the ~2- to 3-km outcrop belt, rules out river bars as an interpretation of the clinoform sandstones.

The migration of fluvial and eolian transverse dunes can produce large-scale patterns of inclined bedding in sands (25–27). In this scenario, striations observed in map view would represent intersections of dune lee deposits with the surface. It is unlikely that transverse dunes could maintain such linear striation trends over an along-strike distance of several kilometers. Transverse dunes typically evolve into structures with considerable sinuosity in their crest lines, and hence they produce foresets with variable dip azimuths (28–30). Even linear dunes typically have planform sinuosities and superimposed bedforms or other features that produce variable dip azimuths where the dunes migrate laterally (27). Furthermore, the migration of dunes produces erosional truncation surfaces that separate sets of climbing dunes. The absence of such surfaces suggests that migrating bedforms, either eolian

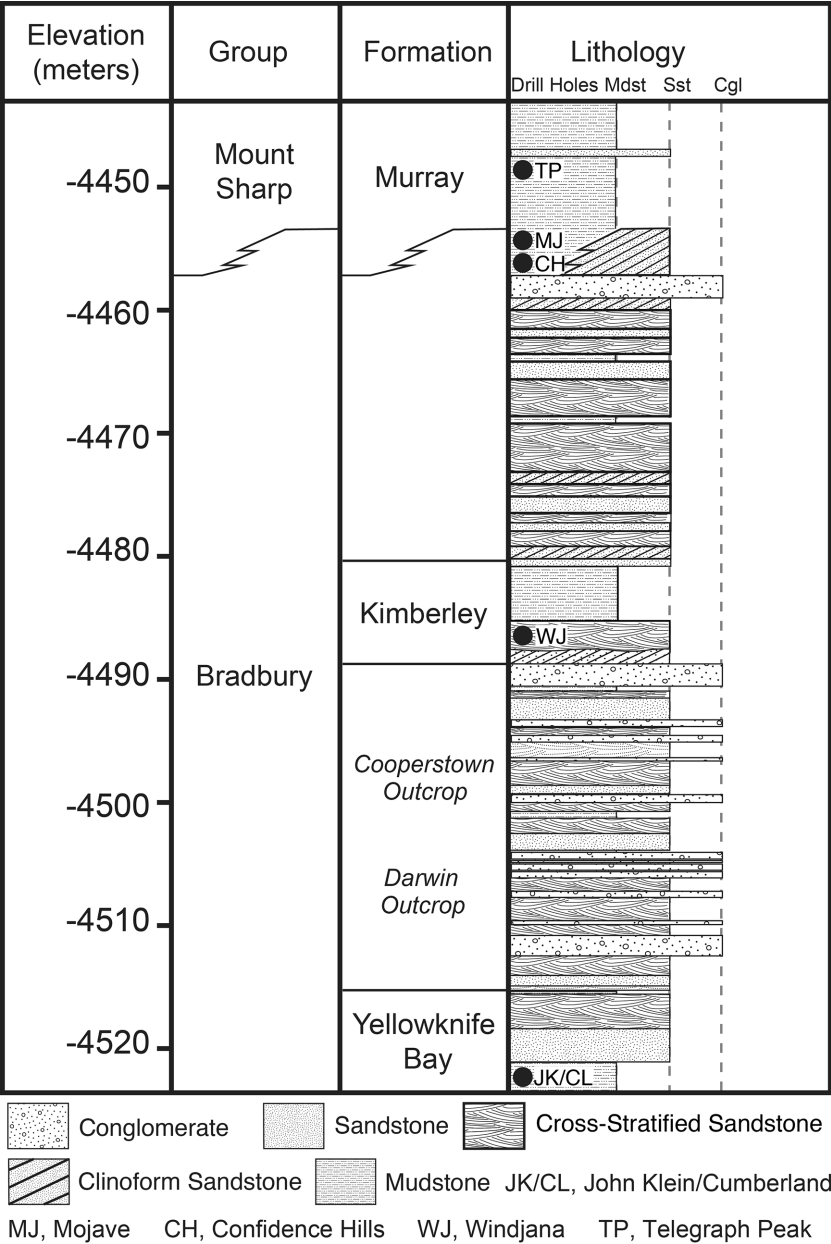


Fig. 2. Stratigraphic column for the sedimentary facies from Yellowknife Bay to Pahrump Hills. The contact between the Bradbury group (Aeolis Palus) and the Mount Sharp group (Aeolis Mons) is marked by interfingering of facies. Mdst, mudstone; Sst, sandstone; Cgl, conglomerate.

or fluvial, were not responsible for clinoform development in the OSO strata.

Our favored interpretation is that the clinoforms represent deposition at small deltas where braided river mouths entered a body of standing water (31, 32). As a river flows into standing water, sediment transport capacity and competence abruptly decrease, leading to deposition and the formation of deltas (33). Progressive accretion of sediment leads to the formation of inclined foresets dipping systematically in one direction. Thus, we interpret the south-dipping foresets of the clinoform sandstones to result from the southward advance of deltas fed by streams from the crater rim to the north (Fig. 3,

D and E). The limited spread in the trend of delta foresets differs from that of younger terrestrial alluvial systems, which are characterized by a wider spread in the orientation of terminal distributary channels (and thus foresets) due to mud deposition and bank stabilization aided by vegetation (34, 35). However, it is consistent with alluvial landscapes on Earth before the advent of bank-stabilizing vegetation. For example, the Precambrian stratigraphic record shows evidence for the coalescence of individual delta lobes to form a broad complex that might have an arcuate shape across tens of kilometers, but that has an approximately linear delta front at a scale of 100 m to a kilometer (36, 37). A relatively narrow spread

of paleocurrent indicators in distal fluvial facies is consistent with relatively straight shorelines on the Precambrian Earth (38–40). For these older landscapes, which make better analogs for fluvial sedimentation on Mars, the trend of clinoforms would show a narrow dispersion, similar to the martian clinoforms.

This interpretation predicts that southward (downstream), in the region marked today by the lower slopes of Mount Sharp, fluvial-deltaic deposits should undergo a lateral facies transition into fine-grained lacustrine deposits. The southward rise in clinoform elevation can be explained in this context (Fig. 3E). Because they were formed at deltas, the tops of clinoforms represent lake-level elevation, and so the occurrence of the clinoforms at progressively higher elevations requires a net lake-level rise. The clinoforms' small size (a few meters) suggests that water depths at the sites of their development were not deeper than a few meters to a few tens of meters; this would have required substantial infilling of the Gale crater lake basin with sediment, thus leading to aggradation of the fluvial system, in addition to progradation. We propose that large-scale progradation and aggradation of the fluvial system, concomitant with basin infilling, led to the systematic stratigraphic rise of multiple clinoform units (Fig. 3D, E). Clinoform outcrops at different elevations do not represent the same stratal unit but rather distinct units that were stacked during fluvial progradation, which is also commonly the case in terrestrial fluvial-lacustrine deposits (41).

The discovery of deltaic deposits in ancient strata that represent sediment progradation toward Mount Sharp changes the way that we think about its development. Although the modern landscape dips northward away from Mount Sharp, these ancient sedimentary deposits indicate that a basin once existed where today there is a mountain.

The relationship between the Bradbury group and the Murray formation

The contact between the Bradbury group and the Murray formation was explored in the valleys that extend from Hidden Valley to the Pahrump Hills (Fig. 5). The Murray formation is a fine-grained laminated mudstone, based on Mars Hand Lens Imager (MAHLI) observations collected where Curiosity drove into Hidden Valley. This fine-grained, finely laminated facies is overlain by a thickly laminated sedimentary facies with distinctively even layering (Fig. 6). The thickly laminated facies is interstratified with thin-bedded sandstones and is ultimately overlain by crossbedded or clinoform sandstones. Observations of other valley walls show that similar systematic progressions of facies occur at different elevations, with a southward rise in the crossbedded and clinoform sandstones (Fig. 5). We interpret this systematic variation to represent north-to-south interfingering of the coarser, fluvial-deltaic facies of the Bradbury group with the finer-grained facies of the Murray formation. This is consistent with a downstream termination of fluvial influence related to alluvial fans prograding southward from the northern Gale crater rim. In bulk, the Murray formation

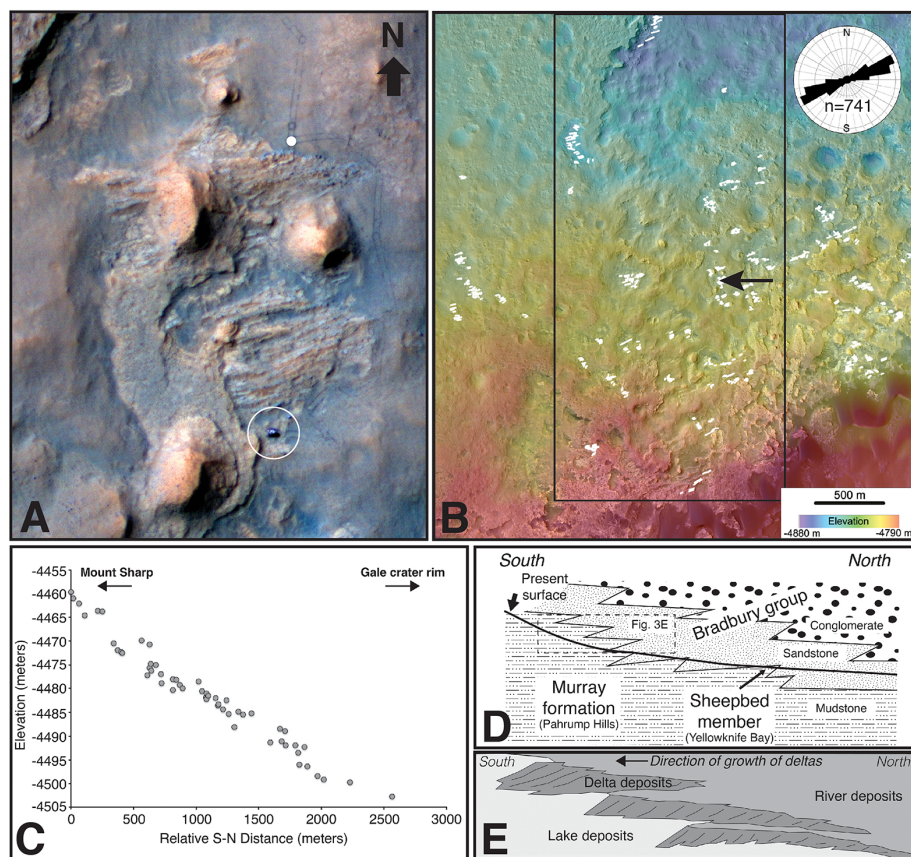


Fig. 3. Occurrence of OSO. (A) OSO as observed in HiRISE image ESP_036128_1755. For scale, the Curiosity rover (circled) is 2.9 m long. The white dot shows the viewpoint for the image in Fig. 4A. (B) Topography of a portion of Aeolis Palus with plotted occurrences of OSO (in white), which rise in elevation to the south. The orientation of striations observed at all mapped localities (inset rose diagram) shows a preferred northeast-southwest orientation; this trend is approximately normal to the inferred south-southeast fluvial paleoflow direction. The box indicates the area sampled to create (C). The black arrow indicates the Kimberley outcrop shown in (A). (C) The southward rise in OSO elevation. Average elevation data for each mapped occurrence within the box in (B) were collapsed onto a single line at the center of the box, and their elevation values were plotted as a function of distance along that line. (D) Facies transition from conglomerate to sandstone to mudstone, in cross section, relative to the rover traverse. This rise in elevation is consistent with rover data; initially, the rover drove uphill across exhumed fluvial facies, then crossed a boundary into deltaic facies (Kimberley region), and ultimately crossed into time-equivalent lacustrine facies (Murray formation at Pahrump Hills). (E) Facies transition from alluvial to deltaic to lacustrine, in cross section, with stacked deltaic clinoforms similar to those observed in Bradbury group. Such facies changes are common on Earth for similar environments but require infilling of the basin at the same time as deltaic progradation occurs.

is believed to overlie the Bradbury group as shown in Fig. 2; however, near the contact, the two units interfinger, so that the Bradbury group locally overlies the Murray formation in the Hidden Valley–Amargosa Valley area.

Interfingering of the Bradbury group and the Murray formation is additionally supported by observations collected farther to the southwest (Fig. 5). Southward-dipping clinoform facies occur in several locations: the entrance to Amar-

gosa Valley at Jubilee Pass, the eastern wall of the Nopah Range, and the northern margin of Panamint Butte. The latter locality is notable in that fine-grained, recessive deposits of the Murray formation directly overlie coarser-grained, south-

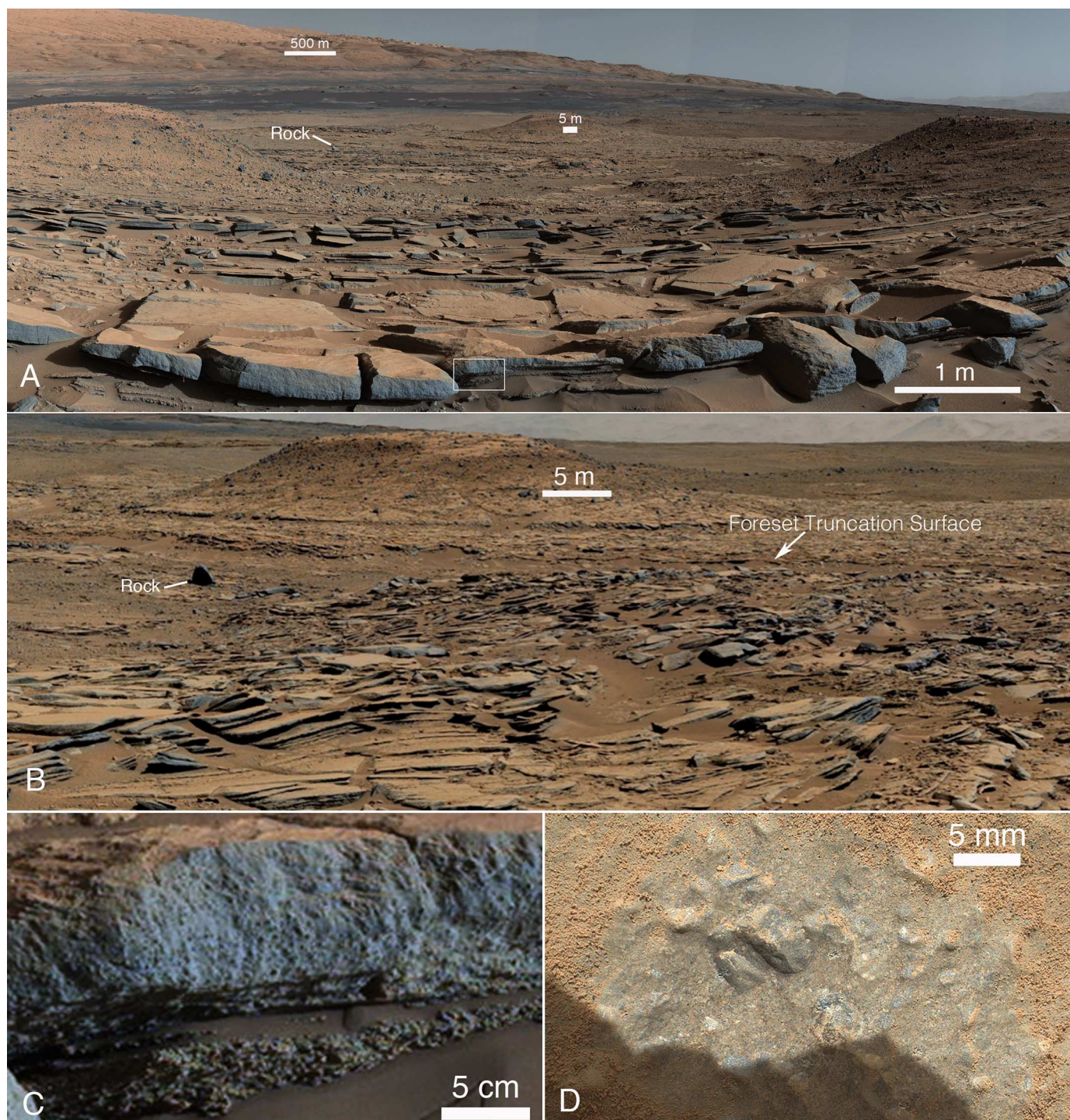


Fig. 4. Clinoform sandstones. (A) OSO at Kimberley; the view is to south and shows south-dipping clinoform facies. The viewpoint is represented by the white dot in Fig. 3A. The box indicates the area shown in (C). “Rock” indicates same feature as in (B) for orientation. The Mastcam image was acquired on martian day (sol) 580. (B) Westward view of the same south-dipping clinoform sandstones in (A). The clinoforms emerge from beneath overlying

strata to the west, exposed at a small butte (the scale bar is placed on this feature). The Mastcam image was acquired on sol 590. (C) Coarse grain size of the clinoform facies. The Mastcam image was acquired on sol 674, at the Square Top locality shown in (A). (D) Close-up of coarse grains observed in clinoform facies at Liga, ~2 m north of the rock indicated in (A) and (B). MAHLI focus merge product 0601MH0003810000203227R00.

dipping clinoform sandstones and conglomerates (fig. S5). The direct juxtaposition of fine- and coarse-grained sediments along a south-dipping contact is strong evidence for the coeval deposition of the Bradbury group and the Murray formation.

Further insight is provided by comparing the two bodies of rock as a function of elevation. Over short distances within the valley system, the two units vary and can be equivalent in elevation. The existence of Bradbury group rocks, including clinoform facies, at the same elevation as laminated mudstone facies of the Murray formation (Fig. 5) suggests temporal equivalence. Alternatively, the rocks might be in unconformable contact; if so, the Bradbury group would be the younger of the two and would have to abruptly about the Murray formation. That relationship would impose substantial constraints on the sediment dispersal represented by Bradbury alluvial fans; the generally southward paleoflow would have to deflect abruptly to either easterly or westerly flow over a short runout distance of less than 100 m. No evidence for this is apparent in the observed sediment transport directions, including the dip of the clinoform sandstones. Therefore, although an abrupt unconformable contact cannot be completely discounted because of intermittent outcrop exposure, the simplest interpretation is that the facies interfinger over a scale of 50 to 100 m, which is characteristic of

fluvio-lacustrine settings (42). In this case, time-lines (stratal surfaces) in the clinoform facies, such as those in the Nopah Range section (Fig. 5), would transition along the inferred paleodepositional slope into mudstones of the Murray formation, exposed at the Pahrump Hills outcrop.

The Murray formation in the Hidden Valley–Pahrump Hills region

After passing through Amargosa Valley, Curiosity arrived at the Pahrump Hills outcrop (Fig. 5). This and smaller outcrops along valley walls constitute the northernmost exposures of the Murray formation, which orbital data suggest extends southward to the hematite-bearing ridge (11) that overlies it in the Mount Sharp group (Fig. 8). Given the rise in elevation from north to south, and assuming subhorizontal bedding, we infer a stratigraphic thickness for the Murray formation of ~150 m between Hidden Valley and the hematite-bearing ridge. The Pahrump Hills section is estimated to be ~13 m thick (Fig. 7A), thus exposing the lowermost ~10% of the formation. (This does not apply to older strata in the subsurface that might be genetically related to the Murray formation.)

Observed in HiRISE image data, the Murray formation has a homogeneous appearance, in part due to the absence of detectable stratification in images with meter-scale resolution. How-

ever, Curiosity image data show that the Murray formation is stratified at the millimeter scale, fine-grained, and exposed in dust-covered outcrops; these attributes promote the uniform appearance observed in HiRISE data.

Four sedimentary facies are recognized within the Murray formation outcrops exposed between Hidden Valley and Pahrump Hills. These include thinly laminated mudstone, thickly laminated mudstone-siltstone, cross-laminated sandstone, and conglomerate. All are exposed at the Pahrump Hills outcrop, where the section begins with conglomerate and is overlain by finely laminated, fine-grained mudstone with a single intercalated bed, 20 to 30 cm thick, of medium-grained, centimeter-scale cross-laminated sandstone (Whale Rock). The top of the outcrop is capped by thickly laminated facies, and massive gray sandstones occur farther uphill, forming a capping unit. The Pahrump Hills outcrop is the reference section for the basal part of the Murray formation, and these rocks, which are broadly exposed in the Hidden Valley–Amargosa Valley system, are designated the Pahrump Hills member (Fig. 7A). Early to late diagenetic textures are also present (supplementary text), including dendritic concretions, prismatic crystal pseudomorphs, and calcium sulfate-filled fractures (fig. S3)

Conglomerate is exposed at the base of the section at Pahrump Hills. Based on the conglomerate's

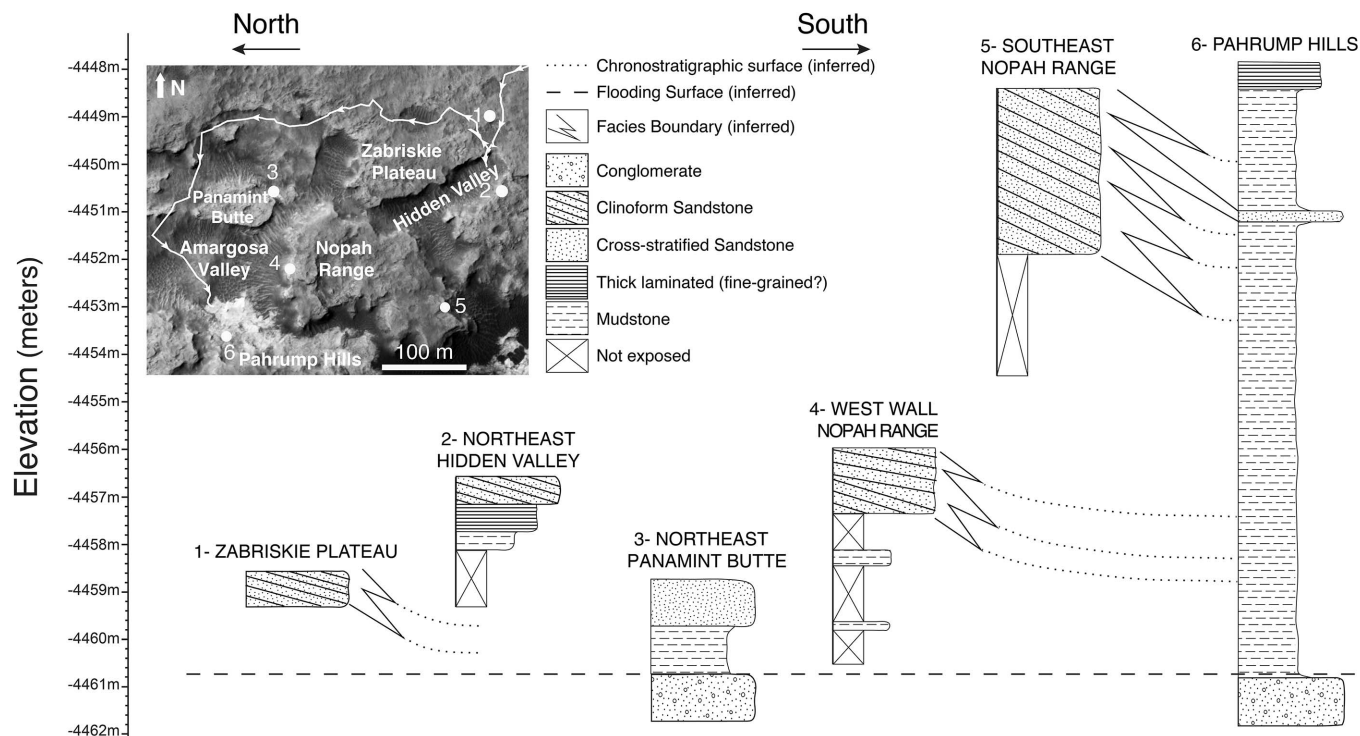


Fig. 5. Facies relationships at the contact between the Bradbury group and the Murray formation. Laminated mudstones are exposed at Pahrump Hills (section 6). Elevation data indicate that these the Bradbury group and Pahrump Hills section could be time-equivalent. The elevations of south-dipping facies in the northerly sections (1 to 5) are lower than those of the mudstones at Pahrump Hills. In Hidden Valley (section 2), laminated mudstones are overlain by what appears to be a gradational, upward-coarsening

sequence that culminates in crossbedded sandstones of fluvial origin (Fig. 6). The lateral transition from coarse-grained fluvial and deltaic facies to fine-grained lacustrine facies is a prediction of the fluvio-lacustrine facies association. This is supported by direct observations at Panamint Butte and Pahrump Hills (sections 3 and 6), where Mastcam data show mudstones overlying conglomerate and sandstone facies. At Panamint Butte, mudstones overlie south-dipping sandstone and conglomerate facies, as shown in fig. S4.

distribution across Jubilee Pass, Panamint Butte, and Pahrump Hills, we infer that it underlies the thinly laminated mudstone facies. This relationship is particularly evident at Panamint Butte (fig. S4). At Pahrump Hills, thinly laminated mudstones overlie and drape depositional topography over the conglomerates.

Finely laminated mudstone is the dominant facies at Pahrump Hills (Fig. 7B). It is also exposed at the Bonanza King outcrop, located along the northeastern side of Hidden Valley. Our analysis constrains the grain size of this facies to be below the 60- to 70- μm limit of resolution (table S1), confirming its classification as mudstone (43). “Mud” is used here in the nongenetic sense, not necessarily implying wetness, and represents only a grain-size designation for which the large majority of particles are below 62.5 μm (43). Stratification of the finely laminated mudstone facies is prominent throughout much of the section (Fig. 7A). Image analysis of representative stratification (supplementary text) shows that the mean lamina thickness is ~ 2.2 mm (figs. S4 and S5 and table S2). The lamination is characteristically parallel, with a horizontal to gently inclined attitude; individual laminae exhibit high lateral continuity of at least several tens of centimeters. An exception to this occurs at the Chinle outcrop, where low-angle truncation surfaces are overlain by laminae that conform to the geometry of the truncation surface. Laminae forming the finely laminated mudstone facies lack evidence of ripples, cross-stratification, or even low angles of climb. Furthermore, no evidence of sediment-filled mudcracks or of intraclasts, which would indicate reworking of muds by currents, is apparent. Additionally, outsized clasts are absent.

The thickly laminated facies is similar to the finely laminated mudstone facies, but it has a characteristically greater lamina thickness (Fig. 7C). This facies occurs along the walls of Hidden Valley at higher elevations, between the finely laminated mudstones and the overlying capping sandstones. It also occurs toward the top of the Pahrump Hills section. It has not been examined by MAHLI, so its grain size is not known; however, Mast Camera (Mastcam) images suggest that it is fine-grained. Image analysis shows that its laminae have a mean thickness of 5 mm, double what was measured in the finely laminated mudstone facies (fig. S5 and table S2). Along the northwest-facing wall of Hidden Valley, the thickly laminated facies is overlain by thicker beds with a more resistant weathering profile, and ultimately by thick-bedded, trough-cross-stratified sandstone (Fig. 6), constituting a thickening-upward succession. There is some evidence that the thickly laminated facies may also transition laterally into thicker, more resistant beds, suggesting coeval deposition.

Cross-laminated sandstone in a single laterally discontinuous bed makes up the Whale Rock outcrop (Fig. 7D). The bed is 30 to 40 cm thick and forms a lens within the overall fine-grained Pahrump Hills stratigraphy. Grains are moderately well sorted and rounded, and the mean grain size is ~ 0.6 mm, reaching up to ~ 0.8 mm. Ripples on Earth generally are composed of finer-grain sediments and only uncommonly include the grain sizes observed in this outcrop. However, for Mars, the ripple stability field expands to include sizes up to 1.3 mm (44). Cross-laminae form beds a few centimeters thick, with subcritical angles of climb and apparent southeastward paleocur-

rent (sediment transport) directions. Several other laterally discontinuous, cross-stratified sandstone beds, also forming lenses, are present at approximately the same stratigraphic level, west of the Whale Rock outcrop. These lenses are all 2 to 4 m in width and a few tens of centimeters in thickness. Trough crossbedding is visible in Mastcam images. The lenses are spaced 5 to 7 m apart.

Synthesis: Reconstructing an ancient lake

Our preferred interpretation for the Murray formation, as it is exposed in the Pahrump Hills area, invokes predominantly subaqueous, lacustrine sedimentation adjacent to a fluvial-deltaic complex. This best explains the attributes of individual facies and the collective assemblage of facies, and it is supported by the broader context of the Bradbury group sedimentology and stratigraphy. The key elements of this model are as follows: (i) The finely laminated mudstones are interpreted as distal deposits related to sediment plumes discharging into a body of standing water, whereas the thickly laminated facies are more proximal equivalents; (ii) the thickening-upward sequence observed in Hidden Valley and at the top of the Pahrump Hills section represents fluvial-deltaic progradation over coeval lacustrine deposits; and (iii) the sedimentology of the facies in the Bradbury group, in particular the clinof orm sandstones, predicts the presence of down-gradient lacustrine equivalents in a more southerly paleogeographic position, thus providing a plausible genetic tie between the Bradbury group and the Mount Sharp group.

The finely laminated mudstone facies, considered jointly with the thickly laminated facies,

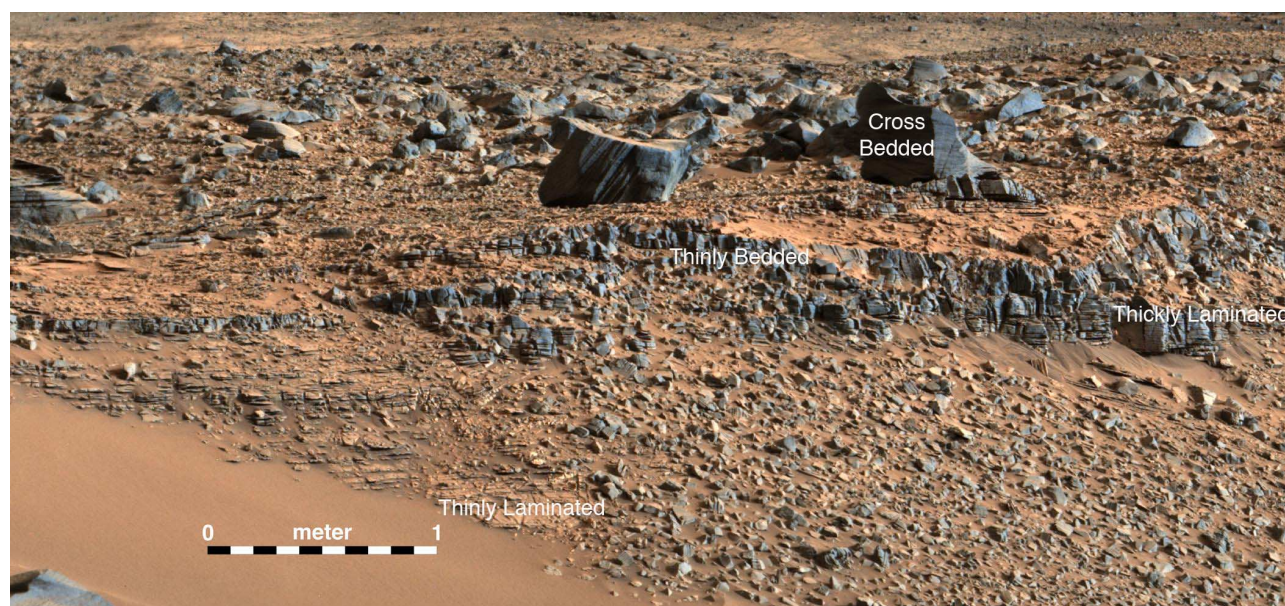


Fig. 6. Stratigraphy of the southeast wall of Hidden Valley. The inset map in Fig. 5 shows the location of Hidden Valley. Finely laminated mudstones exposed at lower elevations grade upward into thickly laminated facies, then into thinly-bedded resistant facies, and ultimately into abundant loose blocks of crossbedded sandstone. Strata of the thin-bedded interval appear to transition laterally into the more recessive laminated mudstones. Although the crossbedded facies at this location is represented by blocks, the blocks can be traced laterally into continuous bedding just a few tens of meters away. This succession is most simply interpreted as a lacustrine facies overlain by laterally prograding fluvial-deltaic deposits.

represents either hyperpycnal or hypopycnal sediment flows (45). Hyperpycnal flows are gravity-driven, slope-hugging currents in front of a delta, which result from the excess negative buoyancy of the discharging sediment plume relative to the ambient lake water. In the hypopycnal case, the plume is positively buoyant and extends across the surface of the lake, until flow expansion diminishes its momentum, resulting in deceleration to the point at which gravity-driven settling drives particles downward through the water column. Thin lamination with regular spacing and substantial lateral continuity is consistent with either mechanism. For moderate-sized rivers, either flow type would create a deposit that thins distally over scales of a few hundred meters (46), thus explain-

ing the difference in lamina thickness between the two facies.

The general absence of coarser graded beds, or beds with flow-deceleration sequences, suggest that deposition may have occurred dominantly by hypopycnal flows. However, the lens-shaped cross-laminated sandstone bodies (Whale Rock and the laterally adjacent sandstones) could be gullied delta foreslope deposits. Their limited east-west extent suggests a north-south elongation of sandstone bodies that is in turn consistent with the general southward transport direction observed throughout the Bradbury group. The climbing-ripple cross-stratification at Whale Rock supports sediment transport under decelerating flow conditions, such as those that

might occur in a bottom-hugging sediment gravity flow. Alternatively, these sandstones might represent fluvial deposits incised into a drying lake bed.

The finely and thickly laminated facies show repeating characteristic lamina thicknesses, similar to varves observed in glacial lake sediments (47). Although the grain size is too fine to confirm, the accentuation of the Pahrump Hills laminae by weathering may be due to repeating differences in grain size, porosity, composition, or grain orientation; such variations may reflect the characteristic time scales of a range of local processes, including the production and delivery of detrital sediment from the catchment area, or precipitation of authigenic materials from the overlying water or within the sediments. Pro- and periglacial lakes on Earth typically develop varves that reflect a combination of seasonal variation in each of these processes. Varves record strong physical weathering in sediment-source regions and where sediment fluxes are driven by seasonal changes in runoff (48). Varves are typically normally graded, and fine particles may reflect the waning stages of hyperpycnal flows or deposition from suspension during intervals when ice covers the water column and prevents wind-driven turbulence. Lamina thicknesses in the Pahrump Hills section are similar to those in fluvial-deltaic depositional systems on Earth (49); consequently, they are consistent with our hypothesis that these laminae record events of distal sediment fallout in a lacustrine setting in the down-dip part of a fluvial-deltaic system.

Alternative mechanisms for the deposition of the mudstone involve eolian processes, including the settling of dust or fine volcanic ash from the atmosphere and traction transport of sand and silt. Wind-blown dust (loess) or ash may constitute some fraction of the sediment in the basin but, if so, the particles are likely to have settled through water. Loess and ash are both characterized by massive bedding, rather than fine lamination, when deposited from the atmosphere (50, 51); the grain-size segregation necessary to create lamination is far more likely to occur if the sediments settle in water. Furthermore, deposition of airborne fine grains cannot explain the regular thickness of the layers or the thinning between the thickly and finely laminated facies. Therefore, we exclude the settling of fine grains directly from the atmosphere as a primary sediment accumulation mechanism.

The observed parallel stratification is not typical of either dry eolian dunes or wet interdunes (52). Flat beds do occur in eolian sandstones, but they generally do so in sets that are a few tens of centimeters thick [and not greater than ~2 m (52)] and are typically interbedded with or grade upward into eolian crossbeds. The sets of flat beds at Pahrump Hills are much thicker and, more importantly, crossbeds with eolian stratification resembling bottomsets and foresets in sandstones on Earth (52, 53) were not observed.

A final set of possible mechanisms includes the formation of eolian impact ripples, “adhesion

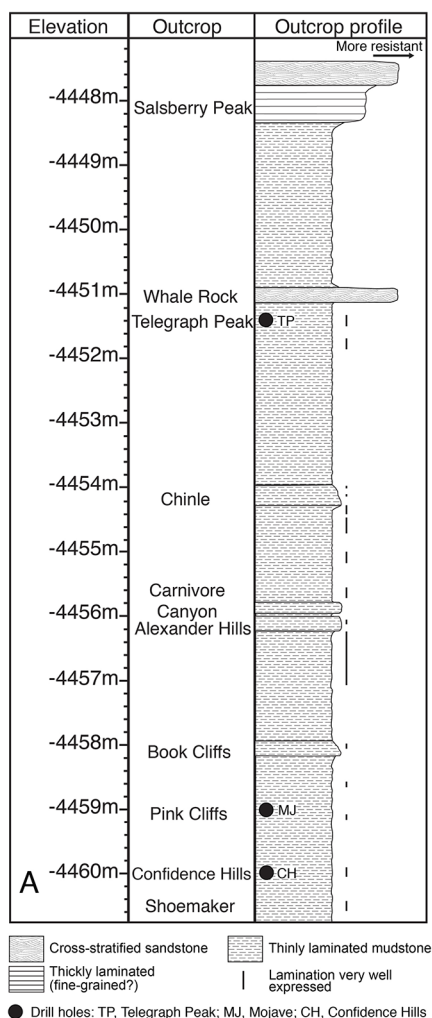


Fig. 7. Stratigraphy and facies of the Murray formation at Pahrump Hills. (A) Mastcam images show that the entire outcrop is finely laminated; in some positions, this texture is particularly well expressed as a result of wind-induced etching and the absence of dust. More resistant intervals probably result from differential cementation. The thinly laminated mudstone has a gradational contact with the overlying thickly laminated facies, which in turn is overlain by cross-stratified sandstone. This thickening- and coarsening-upward sequence mimics that seen in Hidden Valley (Figs. 5 and 6). Whale Rock is an intercalated sandstone lens. (B) Finely laminated mudstone (Mastcam image acquired on sol 792). (C) Thickly laminated facies (Mastcam image acquired on sol 712). (D) Cross-laminated facies observed at Whale Rock, formed by flows in a southeastward direction that created climbing-ripple cross-stratification (Mastcam image acquired on sol 796).

ripples,” and “adhesion lamination;” however, the textures of these features are distinctly different from the lamination observed at Pahrump Hills and are thus discounted (supplementary text).

Exhumation and formation of Mount Sharp

The geologic constraints described above require a major exhumation event as part of the origin of Mount Sharp. Between Yellowknife Bay and Pahrump Hills, Curiosity drove uphill across 75 m of exposed stratigraphy. The fluvial sediments of the Bradbury group, derived from the northern crater rim, were once part of a laterally extensive succession of strata that has been eroded and exhumed (Fig. 8). The net southward transport directions, as well as the north-to-south transition between facies, imply that outcrops of the Bradbury group are erosional remnants of an alluvial plain that stood at least 75 m above the current elevation of Yellowknife Bay, extending southward from the northern crater rim. Furthermore, if the Murray formation is the distal facies equivalent of the Bradbury group, and the Murray formation continues uphill to its contact with the hematite-bearing ridge identified in orbiter data (Fig. 1), then it is possible that those rocks at higher elevations also had northern fluvial equivalents that extended to the crater wall (Fig. 8B). In this case, at least hundreds of meters of erosion would be required to create the topography that is observed today. We therefore interpret the Aeolis Palus surface, and at least the lower part of Mount Sharp’s surface, to be erosional in origin, as represented by the dashed line in Fig. 8.

We suggest the following history as an explanation of our observations: After the formation of the crater and the simultaneous creation of a moat between the rim and the central peak, sediments were supplied via erosion and degradation of the northern wall and central peak (Fig. 8B). Fluvial sediments were deposited along the topographic gradient toward the center of the moat, where a time-equivalent lacustrine facies was deposited. This history is captured in the transition observed between the Bradbury group and the Murray formation (Fig. 1). Deposition of the complete Murray formation then occurred, followed by the hematite-bearing ridge unit, the thin clay mineral-bearing unit, and ultimately a thicker section of sulfate-dominated strata that extends upward to an unconformity, which separates hydrated strata from overlying, apparently anhydrous strata (Fig. 1) (10). The origin of these units is not well known yet, because they have been observed only in data acquired from orbit, but all have interacted with water, and all are assumed to be time-equivalent with the erosion and backwasting of the northern wall and central peak. The unconformity may record a marked change in climate, indicated by the upper strata, a thick sequence of possibly eolian facies (Fig. 8C) (10, 54).

The emplacement of several kilometers of sediment above the Murray formation would have compacted it and any underlying sediments that are currently beneath the surface of Aeolis

Palus (supplementary text). Sediment compaction would have differentially affected the fine sediments of the Murray formation, including those in the subsurface (fig. S7). Coarser sediments adjacent to the rim and peak would have experienced less compaction, and the central peak would have acted as a rigid indenter. This lateral gradient in the degree of compaction would have caused a rotation of younger strata toward the moat center, resulting in shallow dips away from the central peak for strata above the Murray formation (fig. S8). This mechanism may account for the regional dips observed from orbit (55) and provides an alternative to the interpretation that they were formed by mound-building accretion.

After maximum burial and crater infilling, wind-driven erosion resulted in partial exhumation of the crater-filling strata (Fig. 8D). Curiosity landed on nearly the lowest point of the exhumation surface, where fluvial-deltaic deposits were exposed, and then drove uphill to the south and across a facies transition into lacustrine sediments of the Murray formation. A final stage of fluvial activity occurred after exhumation, involving overland flow and sediment transport to create scattered fans (e.g., the Peace Vallis fan), possible deltas (56, 57), and draping strata in the area explored by Curiosity (Fig. 1).

Crater statistics (9, 56) indicate that the exhumation and exposure of most of the hundred to perhaps several hundred meters of strata exposed across Aeolis Palus and at the base of Mount Sharp had occurred by ~3.3 billion to 3.1 billion years ago (fig. S1). Hence, the available evidence suggests that the landscape acquired its present expression by the middle Hesperian Period, and this was followed by the long epoch of very slow eolian erosion that has continued to the present (58). These age constraints require infilling of the crater with sediments to (at least) the level of the top of the Murray formation; burial, compaction, and cementation of those sediments to form rocks; and subsequent erosion and exhumation of those rocks to form the lower reaches of the modern landscape, below the elevation of the hematite-bearing ridge—all within a few hundred million years of the crater’s formation. The erosion rates are estimated to be ~200 m over ~400 million years, or 0.5 m per million years. If the crater was filled with even more sediment, as shown in Fig. 8, then these rates would be higher. In either case, these erosion rates must be adjusted for the time represented by crater infilling and therefore probably represent minimum estimates. We do not yet understand what drove exhumation on early Mars, but the rates indicated by our data are orders of magnitude faster than those calculated for more recent erosion of the martian surface [0.01 m per million years (59)]; they therefore more closely approximate long-term late Noachian to early Hesperian erosion rates for Mars (60) and long-term terrestrial erosion rates for Earth. Given that eroded materials must have been lifted out of the crater to form the existing moat, it seems that wind, rather than water, was the primary agent of erosion. Perhaps higher atmospheric pressure during this early

phase of Mars’s history (61) led to higher surface wind stresses and therefore higher rates of wind erosion (62).

The sediment source of the now-eroded sedimentary rocks was probably the Gale crater rim and walls, which are partly degraded and marked by incised drainage networks (supplementary text). On average, the floor of Gale crater is shallower than expected by 1.9 to 2.1 km, compared with other complex craters of similar diameter (63); this indicates crater infilling or crater rim lowering by this amount (figs. S9 and S10). The inferred amount of crater rim and wall erosion could have supplied more than enough sediment to account for both the Bradbury group and the Murray formation, as blankets of sediment that extended from the crater rim to the central peak (figs. S9 and S10). The current level of rim degradation can be accounted for by ~4.8 km of slope-parallel wall retreat (6.1% of the crater radius), which would generate a sediment layer ~600 m thick on average. Furthermore, an additional hundreds of meters of sediment could be created locally through erosion of the ~1000-km² Peace Vallis catchment, which is one of the few drainages to breach the Gale rim, and which sits directly upslope of the Bradbury group (figs. S9 and S10).

Paleoclimate

Noachian terrains are associated with broad mineralogical and morphological evidence for liquid water, including clay mineral-bearing bedrock, valley networks, open- and closed-basin lakes, and possibly a northern ocean. In contrast, Hesperian terrains provide evidence for water in the form of catastrophic outflows and hydrated sulfates, possibly indicating increased geothermal activity and volcanism. Gale crater formed at 3.8 billion to 3.6 billion years ago (6), toward the end of the Noachian Period, after the formation of the hemispheric dichotomy but perhaps coeval with the formation of the Tharsis bulge on the opposite side of the planet. Crater counts on various surfaces within Gale crater suggests that the crater-filling strata described here were deposited over a few hundred million years, extending into early Hesperian time (6, 9, 56).

Whereas the planetary-scale geomorphic influences on climate largely had arrived at their terminal states when Hesperian stream and lake systems at Gale crater were active, other influences were still evolving. Substantial enrichment in heavy isotopes is evident in multiple atmospheric species, including argon, carbon and oxygen in CO₂, and hydrogen and oxygen in water vapor (64–66). These results point to an initial atmospheric mass and water inventory in Mars’s secondary atmosphere that were a few to many times greater than their present-day values. Mars’s obliquity is poorly constrained during this time period, but it may have been large on average [mean value of 41.8° (67)]. Therefore, the latitudinal distribution of solar forcing, and thus climate and weather, are likely to have varied during this time period. Volcanism and impacts may have dramatically, if only temporarily, forced the climate during this era (68, 69).

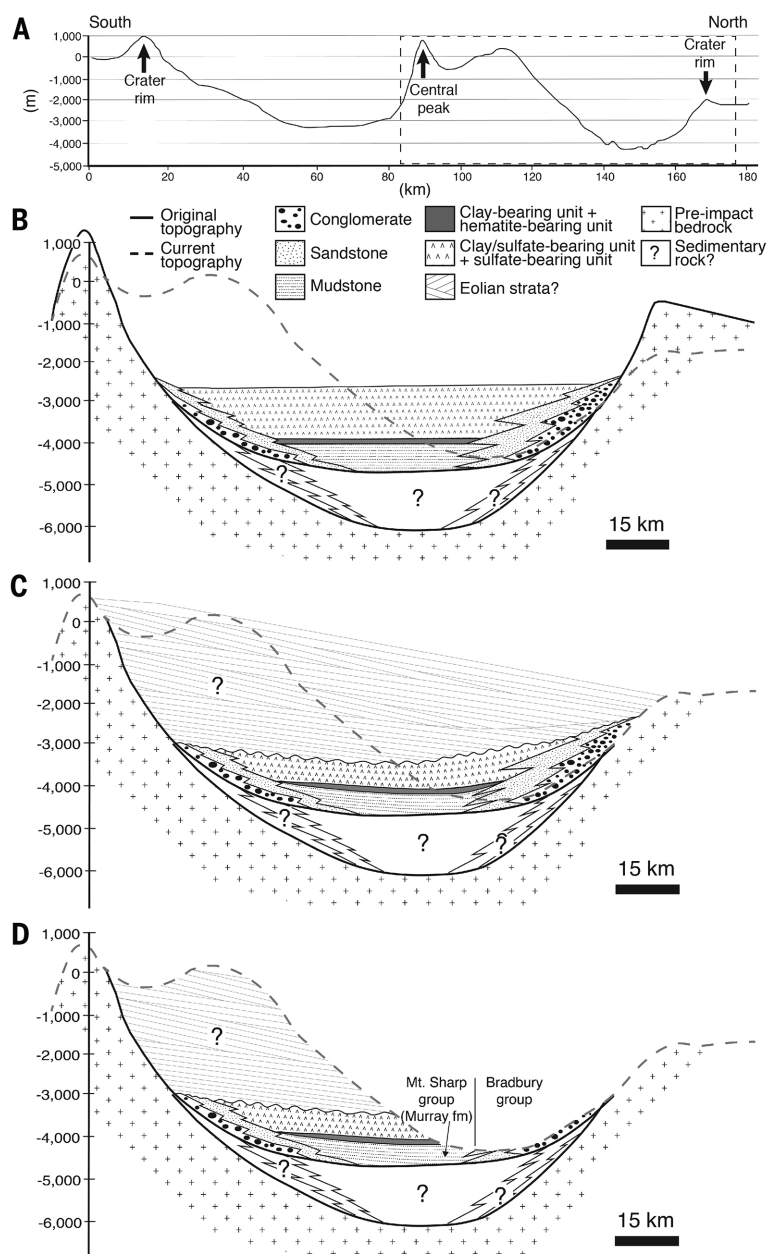
Yet even with an atmosphere substantially enhanced in CO₂, water, and other greenhouse gases derived from volcanic outgassing, models of Mars's climate (70–72) cannot reproduce global average temperatures that approach 273 K, which would make a straightforward case for abundant and geologically long-lived liquid water at the surface. In the absence of long-lived, globally warm temperatures, is regional or transient warming consistent with the geological record? Others have addressed this question for the martian valley networks and paleolakes, arguing that transient impact-induced warming or local meltwater from an icy Mars may have been capable of forming these features. For example, the Icy Highlands Scenario (70) posits that a cold but thicker atmosphere would glaciate the highland terrains but supply meltwater in volumes

consistent with those needed to carve valleys and fill paleolakes.

In light of these cold climate scenarios, the absence of glaciogenic sedimentary deposits or fabrics in the Bradbury group and especially the Murray formation is notable. Coarse cobble or boulder conglomerates formed from tills have not been observed; frost wedges, indicative of strong oscillations in surface temperatures (73), are apparently absent. The well-exposed cross sections of strata exposed at Yellowknife Bay and in the valley systems at Dingo Gap, Kimberley, Hidden Valley, and Amargosa Valley do not reveal features that could be interpreted as frost wedges. Most importantly, well-laminated sediments of the Murray formation at Pahrump Hills form an ideal medium for frost wedges to be preserved, but none have been observed to date.

Even if the Murray formation represents dry eolian sediments, formation of frost wedges would still be expected, as has been the case for extreme glacial events in dry environments on Earth (73). Finally, glacial dropstones were not observed in any of the subaqueous laminated facies. The climate recorded by the ~75 m of rock observed so far does not seem to have been frigid; the crater lake basin and its fringes, in ancient times, do not appear to have experienced glaciation or extreme cold. However, the increased influence of the adiabatic lapse rate in a thicker atmosphere may have created colder conditions on the crater rim. This is supported by geochemical data that indicate limited chemical weathering of sedimentary parent materials (74). Seasonal or glacial ice there could have supplied meltwater that transported sediment to the crater floor.

Fig. 8. History of the infilling of Gale crater and its exhumation to form Mount Sharp. (A) A north-south transect of the current topography, featuring the high southern rim, the crater's central peak at nearly equal elevation, the slightly lower false summit of Mount Sharp, and the much lower northern rim. The northern rim of Gale crater is assumed to have this lower elevation because it straddles the dichotomy boundary. The dotted box represents the part of the crater that is illustrated in (B), (C), and (D). (B) Early infilling of the crater during the aqueous period. The dashed line represents the modern topography, and the solid line represents the original post-impact topography, derived from the profiles of other craters of similar size and with central peaks of similar height. The infilling of the crater was driven by erosion of the northern rim and the central peak, which generated sediment that was transported downslope, fining along the way due to hydraulic segregation of grain size. Crater diameter-depth relationships suggest that an additional 1 to 2 km of crater-infilling strata (pattern with question marks) may lie beneath the present-day surface (figs. S9 and S10). (C) During the subsequent dry period, the bulk of Mount Sharp formed via accumulation of eolian facies, very generally defined. The mass of Mount Sharp exerted a gravitational force that caused compaction of previously deposited strata, resulting in rotation of those strata downward relative to the rigid central peak (figs. S7 and S8). (D) Wind-driven erosion resulted in the exhumation of the crater-filling strata. Curiosity landed on nearly the lowest point of the exhumation surface, where fluvial-deltaic deposits were exposed, and then drove uphill to the south and across a facies transition into lacustrine sediments of the Murray formation.



This is supported by deuterium/hydrogen isotope ratios from ancient clays in a Gale lake deposit, which indicate that a global-equivalent layer of water of 100 to 150 m in thickness was present at the time of sediment accumulation (75).

Our in situ geological observations, which indicate a time series of deltaic-lacustrine complexes within Gale crater, raise additional questions for such cold climate models. The 1 to 4 m stratigraphic thickness of each delta deposit implies a body of standing water of at least that depth, which existed over a period of time long enough to accumulate that sediment and to allow delta foreset beds to prograde for at least many tens to possibly hundreds of meters. This implies that each lake was present for a time span on the order of 100 to 10,000 years, based on terrestrial analog rates (16, 76, 77).

These observations imply a minimum duration when each lake was stable both thermally (as liquid) and with respect to net evaporation and subsequent loss of water to colder regions. The latter effect can be overlooked if only volumetric constraints on water are considered (for example, tying the lake's formation to a transient climate event that supplies its initial volume via meltwater). Unless net evaporation is slowed by increased atmospheric humidity, or lakes are resupplied by runoff, a shallow lake can quickly evaporate. Open expanses of liquid water contained in numerous lakes or a hemispheric ocean would maintain atmospheric humidity and potentially allow for an active hydrological cycle.

The rover data allow for oscillations in the areal extent and depth of lakes between the major lake episodes associated with the deltas, including dry periods when eolian processes were dominant. Nevertheless, another key constraint implied by the observations is the total duration over which lake stability was possible, even if intermittent. Again using terrestrial analogs (76), we estimate that the stratigraphy traversed thus far by Curiosity (~75 m) would have required 10,000 to 10 million years to accumulate, and even longer if the ~150-m-thick Murray formation is included. Additionally, even though individual lakes may have come and gone, they were probably linked in time through a common groundwater table. Over the long term, this water table must have risen at least tens of meters to form the lakes that are marked by the deltas that Curiosity observed.

One of the key criteria for planetary habitability is the duration for which water might have been accessible to enable microbial origination and evolution. The Gale crater floor today is the lowest topographic depression for over a thousand kilometers in any direction, including the northern plains. Our results show that water pooled there, in surface and subsurface reservoirs, for a geologically and perhaps biologically relevant period of time.

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SUPPLEMENTARY MATERIALS

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Supplementary Text

Figs. S1 to S10

Tables S1 to S2

References (78–103)

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RESEARCH ARTICLE SUMMARY

STRUCTURAL BIOLOGY

Structure and flexibility of the endosomal Vps34 complex reveals the basis of its function on membranes

Ksenia Rostislavleva, Nicolas Soler, Yohei Ohashi, Lufei Zhang, Els Pardon, John E. Burke, Glenn R. Masson, Chris Johnson, Jan Steyaert, Nicholas T. Ktistakis, Roger L. Williams*

INTRODUCTION: The lipid kinase Vps34/PIK3C3 phosphorylates phosphatidylinositol to yield phosphatidylinositol 3-phosphate (PI3P). Vps34 is important for processes that sort cargo to lysosomes, including phagocytosis, endocytic traffic, autophagy, and cytosol-to-vacuole transport. In mammalian cells, the enzyme also has roles in cytokinesis, signaling, recycling, and lysosomal tubulation.

Vps34 is present in multiple complexes. Complex I functions in autophagy and contains Vps34, Vps15 (p150/PIK3R4 in mammals), Vps30/Atg6 (Beclin 1), and Atg14 (ATG14L). Complex II takes part in endocytic sorting (as well as autophagy and cytokinesis in mamma-

lian cells) and contains the same subunits as complex I, except that it has Vps38 (UVRAG) instead of Atg14. These complexes are differentially regulated in stress responses. In autophagy, PI3P emerges on small tubular or vesicular structures associated with nascent autophagosomes.

RATIONALE: One of the most compelling questions is how the Vps34-containing complexes are organized and to what extent their intrinsic properties contribute to their differential activities in cells. To understand the mechanisms by which these complexes impart differential activities to Vps34, we sought to determine the structure of com-

plex II and to characterize activities of Vps34 complexes on small and large vesicles. Because the complex resisted crystallization attempts, we screened 15 different nanobodies against the complex, and one of them enabled crystallization.

RESULTS: We obtained a 4.4 Å crystal structure of yeast complex II. The structure has a Y-shaped organization with the Vps15 and Vps34 subunits intertwining in one arm so that the Vps15 kinase domain interacts with

the lipid-binding region of the Vps34 kinase domain. The other arm has a parallel Vps30/Vps38 heterodimer. This indicates that the complex might assemble by Vps15/Vps34

associating with Vps30/Vps38. This assembly path is consistent with in vitro reconstitution of complex II and suggests how the abundance of various Vps34-containing complexes might be dynamically controlled. The Vps34 C2 domain is the keystone to the organization of the complexes, and several structural elaborations of the domain that facilitate its interaction with all complex II subunits are essential to the cellular role of Vps34.

We used hydrogen-deuterium exchange mass spectrometry (HDX-MS) to identify localized changes in all four complex II subunits upon membrane binding. We identified a loop in Vps30 (referred to as the “aromatic finger”) that interacts directly with lipid membranes. Our assays showed that complexes I and II had similar activities on small vesicles (100 nm). In contrast, only complex II was active on giant unilamellar vesicles (GUVs) (2 to 20 μm). This activity was completely abolished by mutation of the aromatic finger.

CONCLUSION: The structure, HDX-MS, and functional data allowed us to devise a model of how Vps34 complexes adapt to membranes. The tips of both arms of complex II work together on membranes. The Vps30 aromatic finger in one arm is important for the efficient catalytic activity of the other arm. The conformational changes that we detected may allow the arms to open to accommodate low-curvature membranes such as GUVs and endosomes.

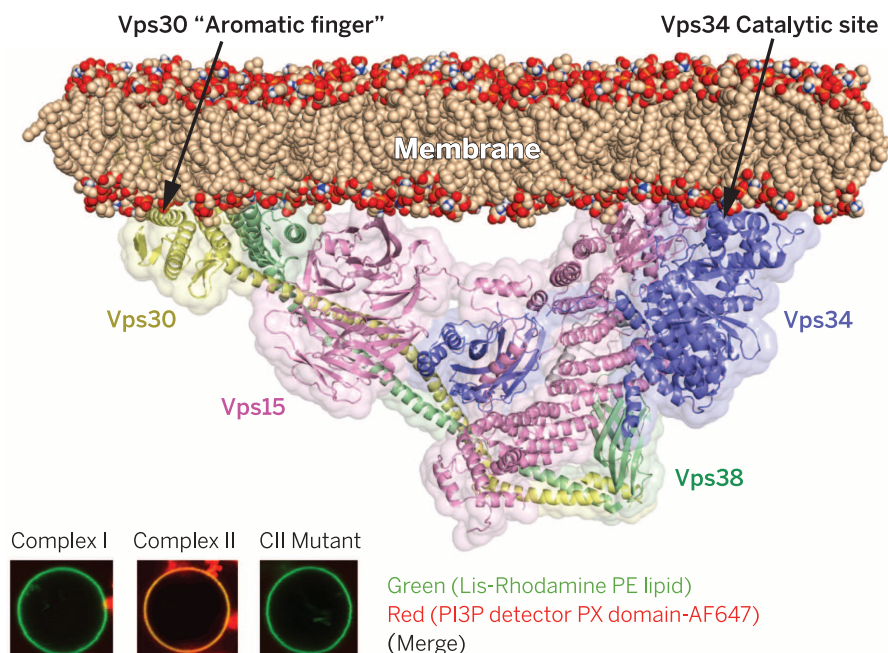
Most of the interactions observed in the complex II structure are likely to be detected in complex I as well. The restriction of complex I activity in autophagy to membrane structures smaller than 100 nm may be related to the inactivity of complex I on GUVs in vitro. ■

The list of author affiliations is available in the full article online.

*Corresponding author. E-mail: rlw@mrc-lmb.cam.ac.uk
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Structure of complex II and its activity on GUVs. In the Y-shaped complex II, the Vps30/Vps38 pair in one arm brackets the Vps15/Vps34 pair in the other arm. Tips of both arms bind membranes. Only wild-type complex II forms PI3P on GUVs; in contrast, complex I and the complex II aromatic finger mutant are inactive. PI3P is detected by a sensor protein (red) binding to GUVs (green). Both complexes I and II have similar activities on small vesicles.

RESEARCH ARTICLE

STRUCTURAL BIOLOGY

Structure and flexibility of the endosomal Vps34 complex reveals the basis of its function on membranes

Ksenia Rostislavleva,^{1*} Nicolas Soler,^{1*} Yohei Ohashi,¹ Lufei Zhang,¹ Els Pardon,^{2,3} John E. Burke,^{1†} Glenn R. Masson,¹ Chris Johnson,¹ Jan Steyaert,^{2,3} Nicholas T. Ktistakis,⁴ Roger L. Williams^{1‡}

Phosphatidylinositol 3-kinase Vps34 complexes regulate intracellular membrane trafficking in endocytic sorting, cytokinesis, and autophagy. We present the 4.4 angstrom crystal structure of the 385-kilodalton endosomal complex II (PIK3C3-CII), consisting of Vps34, Vps15 (p150), Vps30/Atg6 (Beclin 1), and Vps38 (UVRAG). The subunits form a Y-shaped complex, centered on the Vps34 C2 domain. Vps34 and Vps15 intertwine in one arm, where the Vps15 kinase domain engages the Vps34 activation loop to regulate its activity. Vps30 and Vps38 form the other arm that brackets the Vps15/Vps34 heterodimer, suggesting a path for complex assembly. We used hydrogen-deuterium exchange mass spectrometry (HDX-MS) to reveal conformational changes accompanying membrane binding and identify a Vps30 loop that is critical for the ability of complex II to phosphorylate giant liposomes on which complex I is inactive.

The class III phosphatidylinositol 3-kinase (PI3K) known as Vps34 (vacuolar protein sorting 34, encoded by *PIK3C3*) is present in all eukaryotes. It phosphorylates phosphatidylinositol (PI) to yield phosphatidylinositol 3-phosphate (PI3P) (1), which is important for all vesicle-mediated sorting pathways to lysosomes, including processes such as phagocytosis, autophagy, multivesicular body formation, and cytoplasm-to-vacuole targeting. In yeast, PI3P production is totally dependent on Vps34 (2, 3), whereas in mouse embryonic fibroblasts, a VPS34 knockout suggests that 65% of PI3P derives from Vps34, with a class II PI3K producing the remainder (4). Vps34 forms complexes with other proteins (Fig. 1A): Vps15 (encoded by *PIK3R4*, known as p150 in mammalian cells), Vps30 (encoded by *VPS30/ATG6* in yeast, equivalent to mammalian Beclin 1, encoded by *BECN1*) and either Vps38 (UVRAG) or Atg14 (ATG14L). Atg14/ATG14L defines complex I, involved in autophagy, which also includes Atg38 (5) as a fifth subunit (NRBF2 in mammalian cells), whereas Vps38 is characteristic of complex II and essential for vacuolar protein sorting (3). In mammalian cells, complex II is also involved in autophagy, receptor degradation, and cytokinesis (6), as well as

signaling (7, 8), recycling (9), and lysosomal tubulation (10). Independently from complex I and II, Beclin 1 and UVRAG also play separate roles in endosome function and neuron viability (11). Elevated expression of *PIK3C3*, *PIK3R4*, and *BECN1* is associated with more aggressive forms of chronic lymphocytic leukemia (12), consistent with autophagy being involved in tumor maintenance. The crucial roles of complex I and complex II in stress response, nutrition, and disease have led to efforts to develop both activators and inhibitors, such as the recently developed adenosine triphosphate (ATP)-mimetic inhibitors of Vps34 kinase domain (7, 13, 14).

Important questions concerning Vps34 complexes remain, such as the nature of the relationship between Vps15 and Vps34, the roles of Vps30 and Vps38/Atg14 in the functions of these complexes, and how the complexes recognize membranes. To address these questions and assist in the development of complex-specific drugs, we determined the crystal structure of complex II and characterized its dynamics and membrane binding.

The x-ray crystal structure of complex II

Saccharomyces cerevisiae complex II displayed a 1:1:1:1 ratio of four subunits (Fig. 1B). Crystallization required a nanobody (15) that recognized the Vps34 helical domain, as determined by hydrogen-deuterium exchange mass spectrometry (HDX-MS) [residues 386 to 406 (fig. S1)]. Using data from seven native crystals and phases from two Ta₆Br₁₂ derivative crystals (table S1), we produced a high-quality 4.4 Å resolution experimental electron density map (Fig. 1C). Build-

ing the structure was challenging at this resolution. Initial models for several domains of the complex derived from previous structures and distant homologs (16–18) were fitted first, and the remainder of the structure was built directly into the density. The final model consists of 2834 residues out of the 3469 total, with most of the missing residues predicted to be disordered. Although side chains were not visible at this resolution, the sequence register was inferred from previously determined structures for most of Vps34, the WD40 domain of Vps15, and the C-terminal domain (CTD) of Vps30. An approximate sequence register was assigned for the remainder of the structure. The real-space correlation of the model with the density suggests that the fit is reasonable for most of the structure. The poorest fit to the density is in the Vps38 N-terminal C2 domain (fig. S2).

Overall architecture of complex II

The complex has a Y shape, with two long arms and a short hook-like base (Fig. 1, D and E). The base is built entirely of the Vps30 and Vps38 N-terminal domains (NTDs) and coiled-coil 1 (CC1) domains. One of the arms (15 nm in length) consists of Vps15 and Vps34 (Fig. 1E), whereas the other arm (18 nm) includes domains from all four subunits arranged along the Vps30 and Vps38 coiled-coil 2 (CC2). Vps30 and Vps38 show similar architectures except for their NTDs, where Vps38 has a C2 domain, whereas Vps30 is mostly unstructured (fig. S3, A and B). At the C terminus, Vps30 has a BARA domain that binds side-by-side to the CTD of Vps38; we named this domain BARA2. The two arms of complex II correspond to the V shape seen in the low-resolution electron microscopy (EM) structure of complex I (19). It is thus likely that most of the details seen in complex II are preserved in complex I.

Vps15/Vps34 catalytic heterodimer

Vps34 and Vps15 intertwine in an antiparallel fashion, with each of the three domains of Vps15 [kinase and helical (KINHEAT) and WD40] interacting with at least one domain of Vps34 [C2, as well as helical and kinase (HELCAT)] (Fig. 1D and fig. S3, C and D). This network of interactions explains the codependent relationship of the two proteins: Vps34 is essential for Vps15 integrity (3), whereas Vps15 is necessary for Vps34 membrane recruitment and activity in vivo (20).

The N lobe of the Vps15 kinase domain lies at the tip of the right arm and interacts with the C lobe of the Vps34 kinase domain (Fig. 2A). It is not certain whether Vps15 is an active kinase or a pseudokinase. Wild-type (WT) Vps15 is phosphorylated, whereas kinase-dead variants are not, which suggests that Vps15 autophosphorylates (21). Vps15 exhibits nontypical residues in critical catalytic elements: a 145-His-Gly-Asp sequence motif instead of His-Arg-Asp in the catalytic loop, a 165-Asp-Phe-Ala sequence motif instead of Asp-Phe-Gly in the activation segment, and the absence of a GxGxxG motif (G, Gly; x, any amino acid) (P loop) that normally binds phosphates in ATP (22)

¹Medical Research Council (MRC) Laboratory of Molecular Biology, Cambridge CB2 0QH, UK. ²Structural Biology Research Center, VIB, B-1050 Brussels, Belgium. ³Structural Biology Brussels, Vrije Universiteit Brussel, B-1050 Brussels, Belgium. ⁴The Babraham Institute, Cambridge, UK.

*These authors contributed equally to this work. †Present address: Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada. ‡Corresponding author. E-mail: rlw@mrc-lmb.cam.ac.uk

(fig. S3C). Although these substitutions are quite rare in the human kinome, they are found both in active kinases and pseudokinases (23). The Vps15 kinase domain in our structure is probably in an inactive conformation because the long, partially ordered activation loop intrudes into the ATP binding site and would thus prevent ATP binding (Fig. 2B).

The central part of Vps15 forms nine helical pairs wound into a right-handed helical solenoid stretching from the Vps15 kinase domain to the Vps38 CC1 and CC2 domains (Fig. 1D). The first eight helical pairs are HEAT-type repeats. The helical solenoid has a concave face that nestles Vps34 C2. The Vps15 solenoid is followed by four more helices in a bundle (residues 788 to 885) capping the hydrophobic end of the solenoid. Most of the Vps15 linker between the solenoid and WD40 is disordered (residues 889 to 985),

except for an extended section (residues 986 to 1050) threading along the surface of Vps34 C2.

The CTD of Vps15 is a seven-bladed WD40 domain (16) that bridges the Vps15/Vps34 and the Vps30/Vps38 pairs. On one side, conserved residues in the WD40 β 3/ β 4 loop of blade 4 (1261-Arg-Phe, referred to as the RF loop) reach Vps34 C2, whereas on the other side, the only two other invariant residues, Glu¹⁰⁷⁷ and Ser¹¹²¹ (which are located between blades 1 and 7), contact Vps38 BARA2. Finally, one surface of the WD40 domain straddles Vps30 CC2. Due to this interaction, the greatest HDX differences in WD40 between the heterotetramer and the Vps15/Vps34 heterodimer are for peptides in blades six and seven, which contact Vps30 CC2 (Fig. 2C). Several Beclin 1 phosphorylation sites map to this CC2/WD40 interface (fig. S4), where they could affect assembly of the active heterote-

tramer. The region of Vps30 in contact with the WD40 domain coincides with a region in mammalian Beclin 1 that is referred to as the evolutionarily conserved domain (ECD) (24). Instead of binding Vps34 directly as previously proposed (24), ECD binds to Vps15 WD40 and, therefore, only indirectly to Vps34.

Vps15 as a potential regulator of Vps34

Several catalytically important elements of Vps34 contact the kinase domain of Vps15: the activation loop (residues 749 to 767), helix α 10, and the α 11/ α 12 elbow (residues 860 to 864) (Fig. 2A). These interactions are consistent with the observation that deletions in the α 11/ α 12 region prevented association between Vps34 and Vps15 (25). In protein kinases, the dynamics of the activation loop regulates catalytic efficiency (26). The proximity of Vps15 could modulate the active

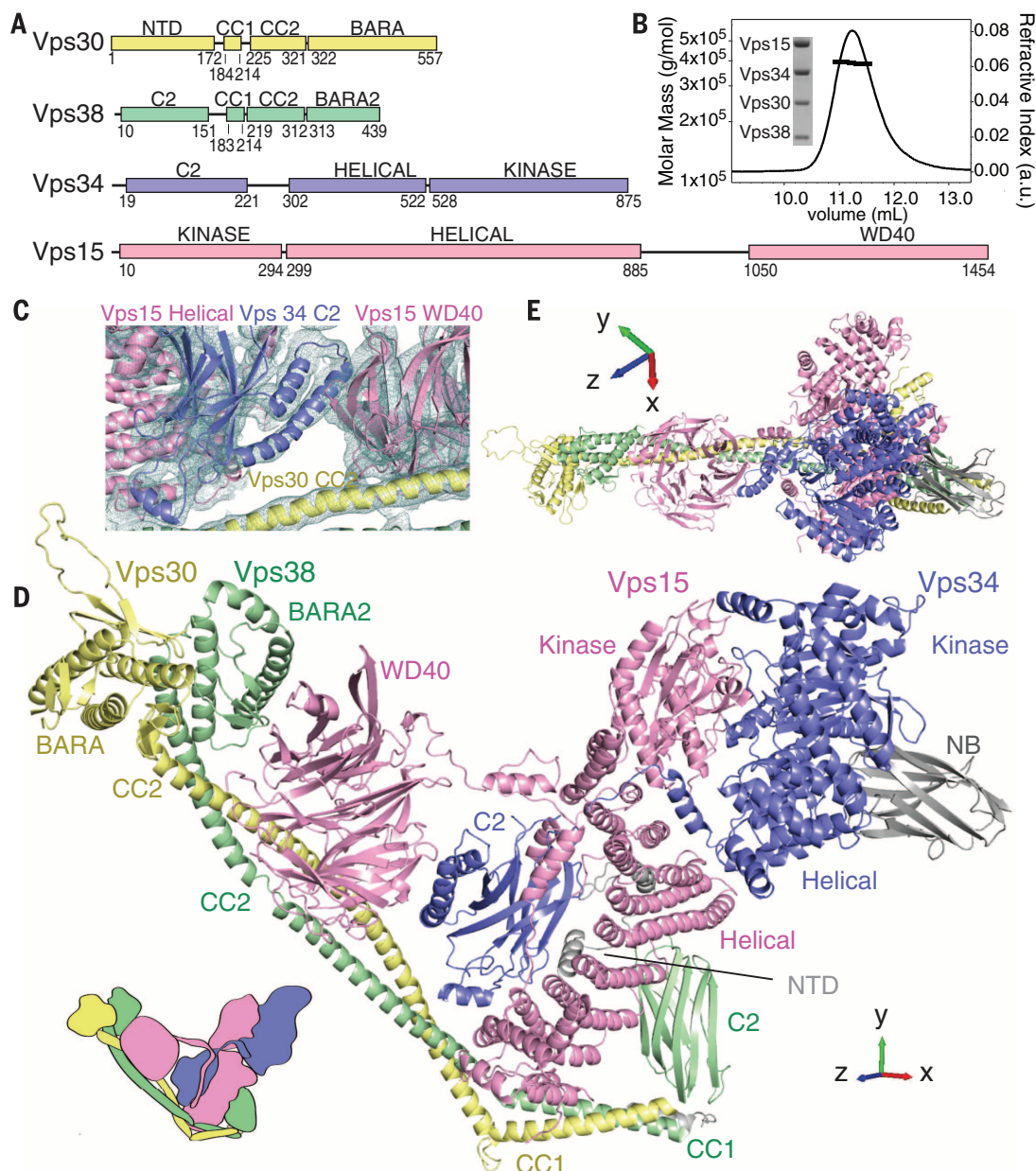
Fig. 1. Complex II structure.

(A) Domain organization of complex II subunits.

(B) Multiangle light scattering (MALS) and SDS-PAGE analyses of complex II show a 390-kD heterotetramer with 1:1:1:1 stoichiometry. a.u., arbitrary units.

(C) Experimental electron density contoured at 1.1 σ for part of the model.

(D) Complex II has a Y shape with two arms and a base. NB, nanobody. (E) Rotated view of the complex.



conformation of the Vps34 lipid-binding site, including the activation loop. SUMOylation of residue Lys⁸⁴⁰ in helix α 10 of hsVPS34 increases kinase activity (27). This modification could regulate both the conformation of the Vps34 activation loop and its interaction with Vps15.

Vps15 could influence Vps34 in multiple ways: stabilizing the enzyme, inhibiting basal activity by restricting the activation loop, and contributing to membrane recruitment. The roles of Vps15 would be akin to the multiple roles of regulatory subunits of class IA PI3Ks. Oncogenic mutations of class I PI3Ks unmask the inhibitory role of the regulatory subunit (28). In a human metastatic melanoma, a Vps15 Arg¹⁰⁷→Gln¹⁰⁷ mutation in the kinase domain (29) is in close proximity to the C2/HELCAAT linker helix and could affect Vps34 activity by reorientating the Vps15/Vps34 kinase domains.

VPS34 C2 domain as a hub for complex II formation

Although each Vps34 domain interacts with Vps15, the most extensive contacts involve Vps34 C2, which rests between the arms of the Y, engaging all subunits (Fig. 3A). This domain was crucial for the function of Vps34 in cells (Fig. 3B) and important for tight association with Vps15 (fig. S5). C2 domains often serve as membrane interaction modules; however, given the dense protein-protein interaction network centered on Vps34 C2, this domain is unlikely to also bind membranes.

The relationship of Vps34 C2 to Vps34 HELCAAT is completely different than what has been observed for class I PI3Ks. The C2 domain of class I PI3Ks interacts extensively with the helical domain, whereas Vps34 C2 is remote from the rest of the enzyme, cradled by Vps15 HEAT (Fig. 3, C and D). This relationship of Vps34 C2 to the Vps34 HELCAAT may imply substantial flexibility around the linker between these two regions of Vps34, as suggested by a relatively high rate of global HDX (fig. S6). Long-range motions of Vps34 HELCAAT have been observed in the EM study of mammalian complexes I and II (19). In our structure, most of the C2/HELCAAT linker appeared disordered. However, a conserved linker helix (Fig. 3C) nestles in a crotch between the Vps15 and Vps34, where it could provide a pivot for Vps34-HELCAAT motions.

In addition to a β -sandwich scaffold, Vps34 C2 deploys two distinct features to assist with its role as a nucleus for complex II. The extended loop between the β 1 and β 2 strands (CBR1) reaches the Vps30/Vps38 CC2 domain (Fig. 3E), analogous to the critical interaction of the equivalent loop in class I PI3K C2 domains with the iSH2 coiled coil of the p85 regulatory subunit (28). This constitutes the only contact of Vps34 with the Vps30/Vps38 unit and explains the previously reported binding of hsVPS34 C2 to a Beclin 1 region encompassing the CC2 domain (30). Deletion of CBR1 resulted in a phenotype similar to that resulting from the deletion of *VPS34* (Fig. 3B), which sug-

gests that this is an important interaction in the complex.

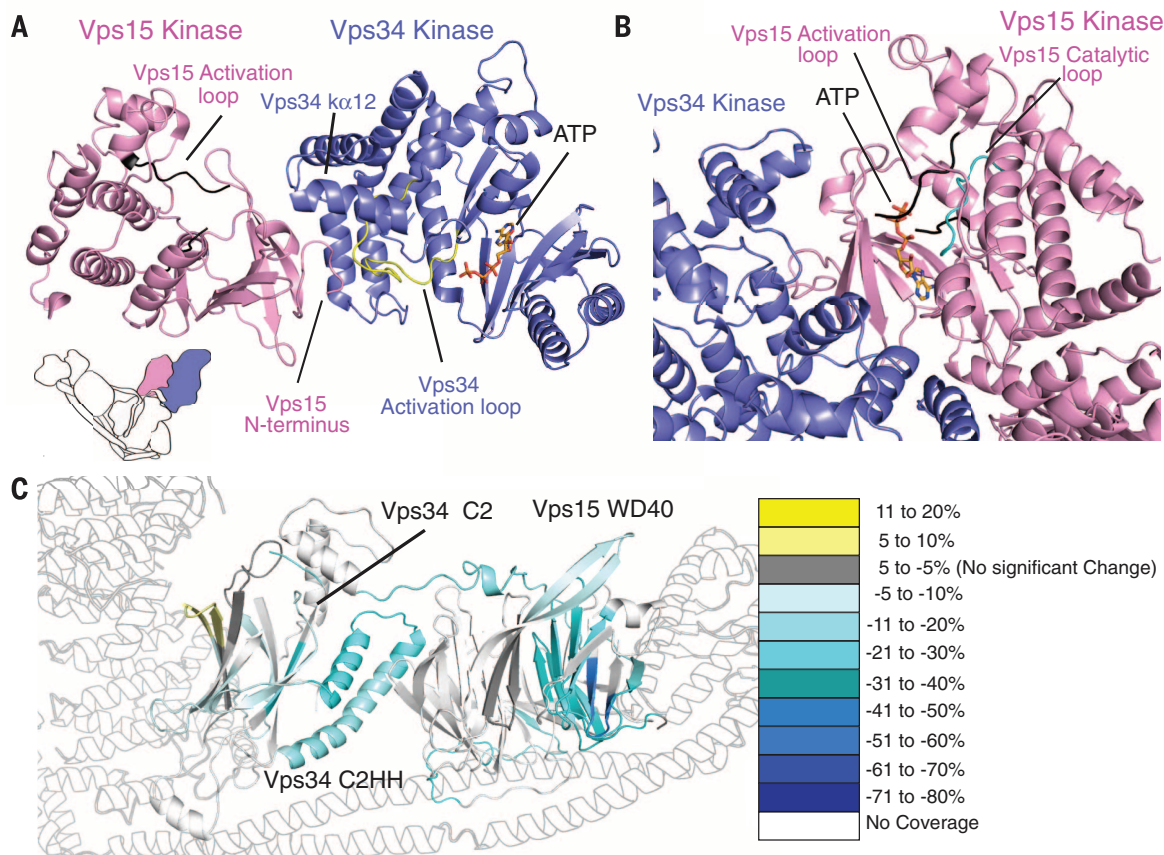
A helical hairpin insertion [C2 helical hairpin (C2HH)] in the β 7/ β 8 loop reaches the RF loop in Vps15 WD40 (Fig. 3A). The HDX rate for C2HH was lower in complex II than in the Vps15/Vps34 dimer (Fig. 2C), suggesting a stabilizing role for C2HH. Nevertheless, the global rate of exchange for C2HH was higher than for the rest of Vps34 C2, indicating flexibility (fig. S6), which might be regulated by phosphorylations (fig. S4). Several of these modifications affect the activity of mammalian VPS34 (31, 32). Deletion of C2HH in yeast Vps34 resulted in a temperature-sensitive phenotype (Fig. 3B).

Organization of the Vps30/Vps38 pair

Vps30 and Vps38 interact along their entire lengths, forming an elongated structure that fits like a bracket around the Vps15/Vps34 heterodimer, along which Vps38 and, to a smaller extent, Vps30 establish multiple contacts with Vps15/Vps34. The two central coiled coils (a short CC1 and a longer CC2) of Vps30/Vps38 form a parallel heterodimer. Based on a low-resolution EM model (19), a parallel arrangement of these two proteins was also proposed for the mammalian complexes I and II, whereas the homodimeric CC2 domain of Beclin 1 forms an antiparallel homodimer (33). The bracket-like organization of the Vps30/Vps38 pair relative to Vps15/Vps34 suggests that the complex might assemble from these two

Fig. 2. The Vps15/Vps34 heterodimer.

(A) The N lobe of the Vps15 kinase domain interacts with the Vps34 activation loop (yellow) and C-lobe helices α 10 and α 12. ATP in Vps34 was modeled based on other lipid kinases. (B) The activation loop (black) in the Vps15 active site would prevent ATP binding (ATP model based on other protein kinases). (C) Vps15 WD40 and Vps34 C2 (including C2HH) peptides display a lower rate of HDX in complex II than in the Vps15/Vps34 heterodimer. HDX for the rest of the complex (gray outline) is not shown.



pairs. This is supported by a pulldown experiment showing that a purified Vps30/Vps38 heterodimer can capture purified Vps15/Vps34 (fig. S7).

The C-terminal Vps30 BARA and Vps38 BARA2 domains are at the tip of the left arm, with Vps30 BARA interacting exclusively with Vps38 and Vps38 BARA2 contacting both Vps30 and Vps15 WD40. Vps30 BARA in complex II agrees closely with the structure of the isolated BARA (17, 34) (Fig. 4A). Whereas Vps30 BARA has three β - α repeats, Vps38 BARA2 has two incomplete BARA-type repeats. The first repeat includes a helix preceded by an extended structure but not forming a clear β sheet, whereas the second repeat is complete (Fig. 4B).

The most conserved part of Vps30 BARA forms an extensive interface with Vps38 BARA2,

which is consistent with HDX data [the Vps30 peptide spanning residues 385 to 389 at the BARA/BARA2 interface being more protected in complex II than in free Vps30 (Fig. 4A)]. The Vps30 NTD, CC1, and CC2 also contribute to interaction with Vps38. In fact, the NTD, C2, and CC1 domains of Vps30 and Vps38 are sufficient to form a stable heterodimer in solution (Fig. 4C).

The NTDs of Vps30 and Vps38 meet at the base of the complex, where they interact primarily with Vps15. The Vps30 NTD is not well defined, but we could build three disconnected helices that may be part of this domain (Fig. 5A). In our interpretation, one of these helices forms a triple-helical bundle with CC1 of Vps38/Vps30. Furthermore, the density assigned to the Vps30 NTD is adjacent to Vps38 C2. This close spatial

relationship is supported by our HDX-MS findings that identified a single nanobody binding to both Vps38 C2 (residues 45 to 55) and Vps30 NTD (residues 63 to 76). The HDX-MS results suggested additional contacts (not apparent at this resolution) between these domains, based on the large protection of the peptide spanning residues 82 to 86 in the Vps30 NTD in complex II versus Vps30 alone (table S2A).

Given that Vps38 defines complex II and is essential for vacuolar protein sorting (17), we used a carboxypeptidase Y (CPY) sorting assay in a *vps38 Δ* mutant to characterize the role of Vps38 C2 and BARA2 (Fig. 5B). Δ BARA2 showed only a partial rescue of CPY sorting, whereas Δ C2 did not rescue CPY sorting due to Vps38 degradation (Fig. 5C). Thus, Vps38 BARA2 is important for vacuolar protein sorting, and Vps38

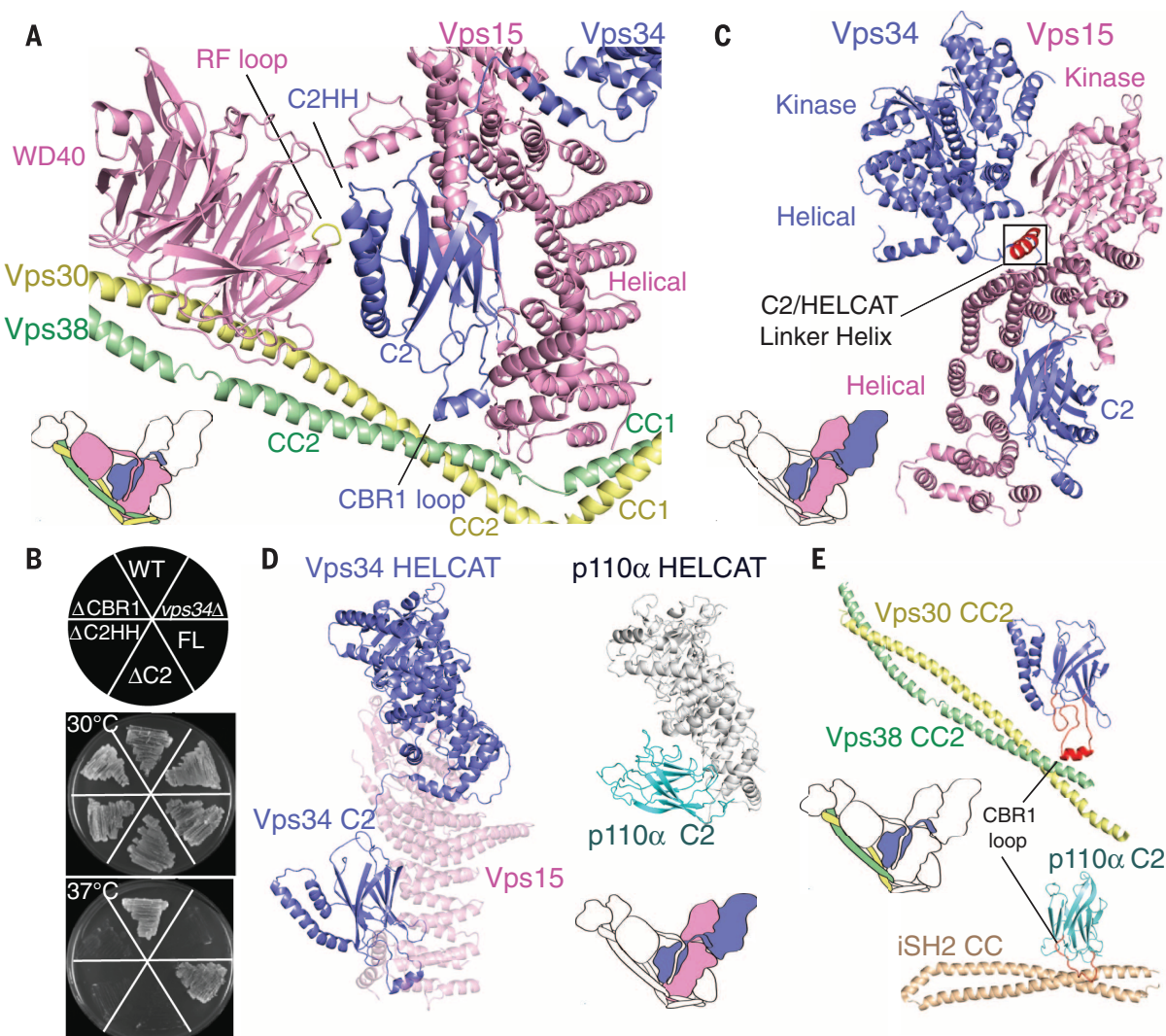


Fig. 3. The Vps34 C2 domain is at the heart of complex II. (A) The C2 β sandwich and its distinct structural features (C2HH and CBR1 loop). (B) Effects of Vps34 C2 mutants on temperature-sensitive growth. Deletion of Vps34 C2 (Δ C2), C2HH (Δ C2HH), or CBR1 (Δ CBR1) prevents growth at 37°C, similar to results for *vps34 Δ* . Full-length Vps34 (FL) grows at 37°C, as does the WT strain. (C) The helix (red) in the Vps34 C2/HELCAT linker

fits between the Vp15 kinase and helical domains. (D) Vps34 C2 does not contact HELCAT (left), in contrast to class I PI3Ks (right). In complex II, Vps15 bridges the Vps34 C2 and Vps34 HELCAT domains. (E) The Vps34 C2 CBR1 loop contacts Vps30/Vps38 CC2. This is similar to the CBR1 loop of class IA PI3Ks contacting the coiled coil (iSH2 CC) of the regulatory subunit.

C2 is essential for the stability of Vps38 and, consequently, for complex II formation.

Implications for complex I

In complex I, Vps38/UVRAG is substituted with Atg14/ATG14L. Although the NTDs of Vps30, Vps38, and Atg14 differ, the overall similarity of their domain organizations suggests that these proteins may have evolved from a common ancestor. The lengths of the Atg14 and Vps38 coiled-coil regions are comparable, which suggests that Atg14 probably interacts similarly with the CC1 and CC2 domains of Vps30 as compared to its interaction with Vps38.

The C-terminal extension of human Atg14L contains a BATS domain previously shown to interact with membranes. In our model, the BATS domain would share proximity with other elements of the complex involved in membrane binding (see next section). In yeast, the amphiphilic helix at the C terminus of Atg14 (residues 240 to 261) could act as its functional counterpart. The absence of a C2 domain in Atg14 to interact with the Vps30 NTD may explain why the Vps30 NTD is dispensable for autophagy, in contrast to the critical role of the Vps30-CTD (17).

Membrane interactions of complex II

Because the activity of all PI3Ks is controlled by their association with membranes, we examined

this interaction for intact complex II. Comparing HDX for complex II in the presence and absence of membranes showed that localized changes in exchange for all four subunits accompany membrane binding (Fig. 6A and table S3). Vps30 BARA (residues 424 to 443) shows a decreased rate of HDX, suggesting that this region directly interacts with membranes, consistent with Vps30 BARA greatly contributing to endosomal localization of complex II (17). The analogous region (359-Phe-Phe-Trp) in Beclin 1 BARA contains the membrane-binding aromatic finger (34). For simplicity, we also will refer to Vps30 residues 430-Phe-Arg-Lys-432 as the aromatic finger (fig. S3A). Two known membrane-binding elements in Vps34, the activation loop and the C-terminal α 12 helix, did not display a decrease in HDX rate. An increased rate of HDX in the Vps34 activation loop likely reflects a disruption of the contact between this loop and the N terminus of Vps15 enabling the activation loop to bind lipids, whereas contacts made by the α 12 helix in the closed conformation observed in complex II are replaced by membrane interactions (18). Thus, the net HDX rate that we observe for the Vps34 kinase domain upon membrane binding is presumably a sum of increases in HDX due to conformational changes and decreases associated with direct membrane interaction.

In our model for Vps34 complexes on membranes (Fig. 6A), the tips of the two arms contact

the membrane: one arm via the Vps34 activation loop, the Vps34 α 12 helix, and the Vps15 N-terminal myristoylation site, and the other arm via the Vps30/Beclin 1 BARA domain. Both Vps15 WD40 and Vps34 interact with the yeast lipidated G α subunit Gpa1 on endosomes (35), and VPS34 interacts with G α i at the midbody (36). It is plausible that membrane-localized Gpa1 would fit into the notch between the two arms of complex II. Mammalian VPS34 complexes are regulated by Rab5 (37) and Rab7 (38), and these G proteins may occupy the same crevice between the two arms of the complexes.

Activity of Vps34 complexes on small and large vesicles

To evaluate the influence of the Vps30-containing arm on Vps34 activity, we compared activities of complex I, complex II, and the Vps15/Vps34 heterodimer on small and large vesicles. On small unilamellar vesicles (SUVs), complexes I and II had similar activities that are higher than that of the heterodimer (Fig. 6B). Complex II with a mutation in the Vps30 aromatic finger (430-Phe-Arg-Lys-432 mutated to Asp-Asp-Asp) showed lower activity, similar to that of the Vps15/Vps34 dimer (Fig. 6B). Notably, complexes I and II differed substantially in their activities on giant unilamellar vesicles (GUVs). Complex I and the Vps15/Vps34 heterodimer were inactive on GUVs, whereas complex II efficiently phosphorylated these relatively low-curvature membranes (Fig. 6C). This ability depended critically on the Vps30 aromatic finger, because the Phe-Arg-Lys/Asp-Asp-Asp mutant had an activity indistinguishable from that of Vps15/Vps34. It is noteworthy that a cluster of posttranslational modifications maps to loops in the Vps30 BARA domain (fig. S4). These inhibitory modifications may prevent the aromatic finger from interacting with membranes. Atg14 might block the basal membrane-binding ability of Vps30 in complex I on GUVs; in cells, this block could be relieved by starvation.

Membrane binding was accompanied by exposure of peptides in both the arms and base of the complex (Fig. 6A), indicating conformational changes compatible with global opening and closing motions such as those suggested by a normal mode analysis (movie S1). These data allow us to devise a model for how complex II adapts to membranes (Fig. 6D). Conformational shifts in the base would enable opening of the arms to adjust to different membrane curvatures. Flexing joints at the junction between Vps30 CC2 and Vps30 BARA and between the Vps15/Vps34 kinase domains would then enhance productive membrane binding. In contrast to complex II, which operates on relatively flat endosomal membranes, complex I is associated with highly curved membranes. Accordingly, human ATG14L has a C-terminal BATS domain that senses membrane curvature (39). Highly curved membranes could more readily fit into the notch between the two arms (Fig. 6E). PI3P recognition and curvature sensitivity conferred by the BATS domain could set up a positive-feedback loop for rapid, localized buildup of PI3P on highly curved membranes,

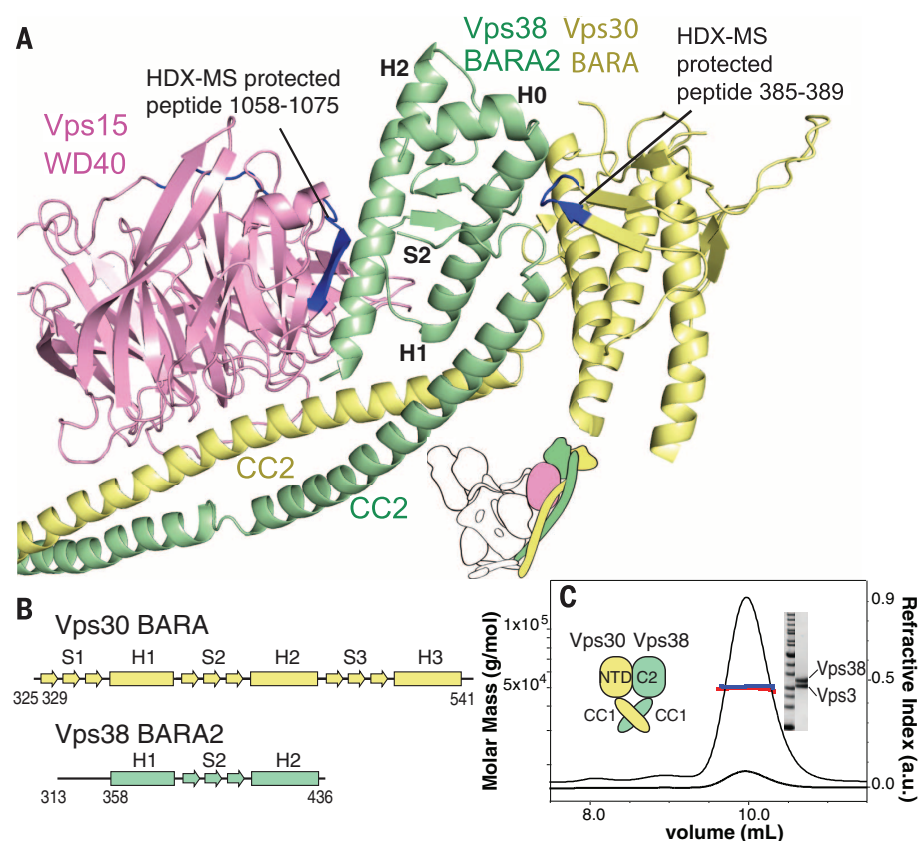


Fig. 4. Organization of the Vps30/Vps38 pair. (A) Vps38 BARA2 bridges Vps30 BARA to Vps15 WD40. (B) Vps38 BARA2 fold is related to Vps30 BARA. (C) MALS results show that Vps30 NTD and Vps38 C2, together with the CC1 regions, form a stable 50-kD complex, as expected for a 1:1 heterodimer.

such as the tubular or vesicular structures associated with the tips of the autophagic isolation membrane (40) originating at the omegasome (47).

The Y-shaped complexes I and II constitute bipartite structures with catalytic activity in the Vps15/Vps34 arm and functional specificity arising from the Vps30/Vps38 (or Vps30/Atg14) arm and base. These assemblies constitute recruitment platforms for regulatory proteins and downstream factors, fine-tuning Vps34 activity. This detailed view of the whole complex provides a framework for designing selective activators and inhibitors of Vps34 complexes targeting not only the active site but also complex-specific membrane- and protein-binding interfaces. The structure should enable a better understanding of the regulation of Vps34 autophagic and endosomal complexes by posttranslational modifications, binding partners, and membranes.

Materials and methods

Constructs

All DNA constructs used in this study are listed in table S5.

Yeast strains and cell growth

Yeast strains used in this study are listed in table S6. Cell growth for protein expression is described in the below sections on protein purification. For cell biology, yeast cells carrying plasmids were grown at 30°C in medium lacking uracil (-URA)

(0.67% yeast nitrogen base without amino acids; 0.5% casamino acids and 2% glucose, supplemented with 0.002% adenine sulfate; 0.002% tryptophan; 0.002% tyrosine; and 0.002% leucine).

Protein purification: Complex II

Plasmids carrying genes encoding *S. cerevisiae* PI3K complex II subunits were transformed into BCY123 or YOY193 yeast cells (see tables S5 and S6 for details). All coding fragments were under the GAL1 promoter (Somatogen). Cells were grown in YM4 medium (0.67% yeast nitrogen base without amino acids; 0.5% casamino acids and 2% glucose, supplemented with 0.002% adenine sulfate; and 0.002% tyrosine) for double-plasmid expression using pRS424- and pRS426-backbone plasmids or in -URA medium for pRS426-backbone plasmids. Flasks containing a total of 10 liters of cells were grown at 30°C in the presence of 2% galactose for 24 hours. Cells were lysed in lysis buffer [50 mM Tris at pH 8.8, 300 mM NaCl, 1 mM dithiothreitol (DTT), 1% Triton-X, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and a protease inhibitor tablet (Roche 05056489001) with glass beads (Sigma G8772)] using a cell disruptor (FastPrep-24, MP Biomedicals). Immunoglobulin G (IgG) beads (GE Healthcare 17096902) were added to the lysate, incubated for 3 hours at 4°C, and then washed once with wash 1 buffer (50 mM Tris at pH 8.0, 300 mM NaCl, 1 mM DTT, 1% Triton-X, and 0.5 mM PMSF) and once with wash 2 buffer (50 mM Tris

at pH 8.0, 300 mM NaCl, 1 mM DTT, and 0.5 mM PMSF). The protein A (ZZ) tags were cleaved by TEV protease in wash 2 buffer overnight. The elution fractions were concentrated and subjected to gel filtration on Superdex 200 16/60 equilibrated in 20 mM Tris at pH 8.0, 300 mM NaCl, and 1 mM tris(2-carboxyethyl)phosphine (TCEP). The aromatic finger mutant complex II (with Vps30 residues 430-Phe-Arg-Lys-432 mutated to Asp-Asp-Asp) was purified in the same way as the WT complex II.

Protein purification: Complex I

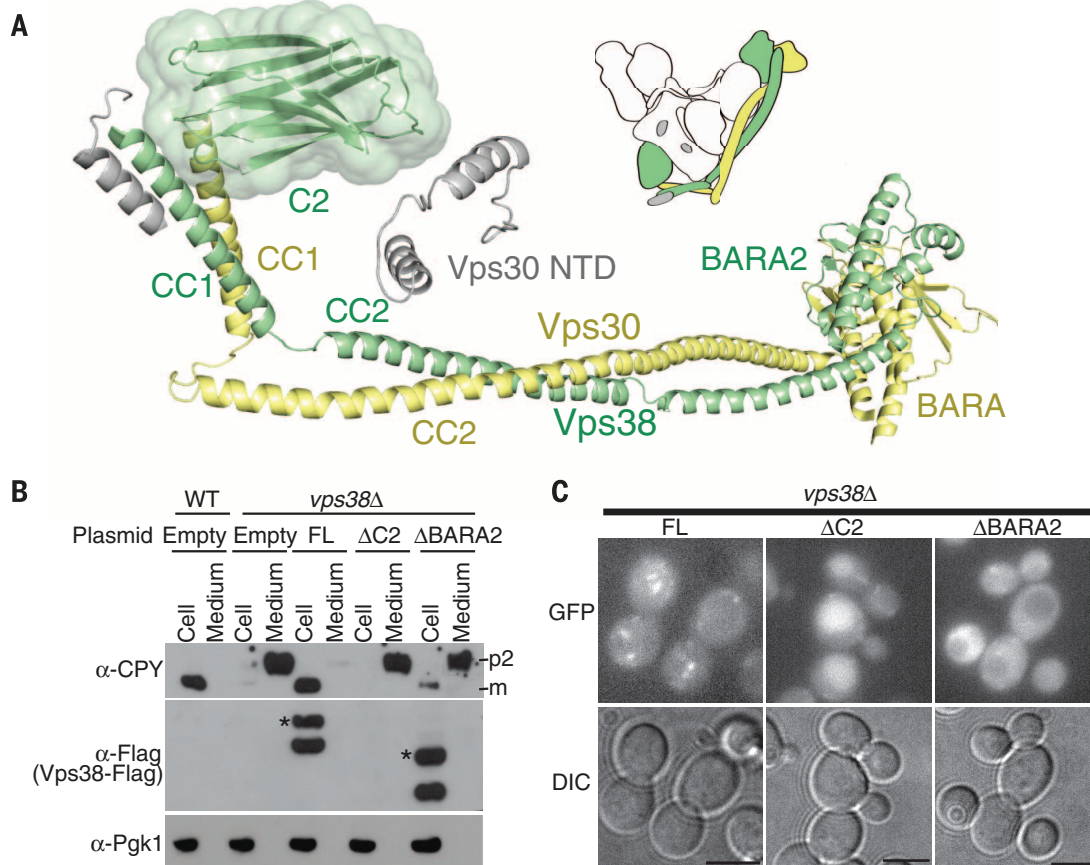
The *S. cerevisiae* complex I was expressed as described above. The protocol for complex I purification was essentially the same as for complex II, with three differences: (i) 150 mM NaCl was used in all buffers, (ii) 0.5% CHAPS was used as a detergent, and (iii) a solution containing 20 mM Tris at pH 8.8, 150 mM NaCl, and 1 mM TCEP was used for gel filtration.

Protein purification: Vps15/Vps34 dimer

The *S. cerevisiae* Vps15/Vps34 heterodimer was expressed as described above. The purification protocol was the same as for complex II, with some differences: Wash 1 and wash 2 buffers contained 500 mM NaCl. Once the protein A tags were cleaved off with TEV protease overnight, the sample was diluted with three volumes of HEP-A buffer (20 mM Tris at pH 8.0 and 0.5 mM

Fig. 5. Vps30 and Vps38 NTDs are important for complex II stability and function. (A) Parallel

arrangement of Vps30 and Vps38 brings the Vps30 NTD close to Vps38 C2. For the Vps30 NTD (gray), a partial model consisting of three helices is suggested. (B) CPY assay in WT and *vps38Δ* strains expressing Vps38 truncations. Protein bands for CPY, Vps38, and Pgk1 were detected with anti-CPY, anti-Flag, and anti-Pgk1 antibodies, respectively. FL Vps38 and Δ BARA2 show two protein bands. Asterisks indicate bands corresponding to Vps38 at the expected size, p2, glycosylated form; m, mature form. (C) Fluorescence microscopy of the *vps38Δ* strain expressing the same truncations as in (B), fused to GFP. FL Vps38 showed a punctate pattern, whereas Δ C2 was in the vacuole, and Δ BARA2 was cytosolic with empty vacuoles. DIC, differential interference contrast. Scale bars, 5 μ m.



DTT) and then loaded on a pre-equilibrated 5 ml Heparin HP column. The protein was then eluted with an NaCl gradient (HEP-B buffer, 2 M NaCl, and 0.5 mM DTT). The kinase-dead vps34 Asp⁷³¹→Asn⁷³¹ (D731N) dimer was expressed and purified as wild type.

Production of nanobodies

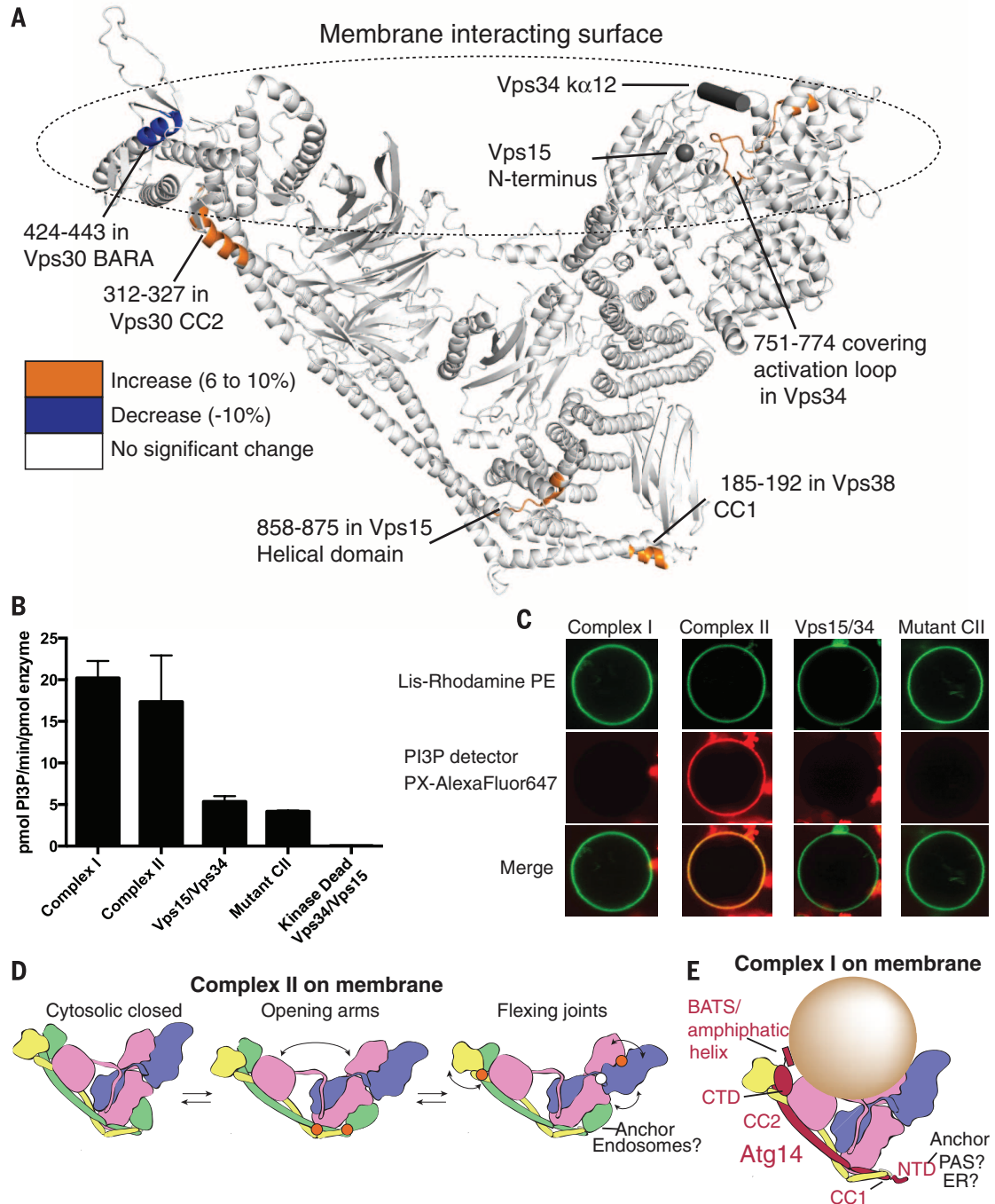
Nanobodies were generated against cross-linked complex II. Protein for generating nanobodies was produced from 10 liters of yeast cell culture. Complex II was purified as described above, except that the wash 2 buffer was composed of 50 mM

HEPES at pH 7.4, 300 mM NaCl, 1 mM TCEP, and 0.5 mM PMSF. Proteins on IgG beads were incubated overnight with TEV protease in 10 ml of wash 2 buffer at 4°C. After the first elution, 5 ml of wash 2 buffer without TEV protease was added and a second elution was performed. The two TEV elution fractions were combined, and CHAPS was added to a concentration of 0.1%. Protein was concentrated for gel filtration (Amicon Ultra 100-kD cutoff, Millipore). Gel filtration was carried out on a Superdex 200 16/60 column (GE Healthcare), which was pre-equilibrated with gel-filtration buffer (20 mM HEPES at pH 7.4, 300 mM NaCl, and

1 mM TCEP). The peak fractions were combined and concentrated to 400 μ l at 6.3 mg/ml (16 μ M). The whole amount was used for the cross-linking. Complex II at 10 μ M was cross-linked using a CovalX K200 stabilization kit (CovalX, Zurich, Switzerland) by following the manufacture's protocol. After removing precipitates by centrifugation, the cross-linked material was frozen in liquid nitrogen and shipped on dry ice to the Steyaert laboratory for immunization.

Complex II-specific nanobodies were generated following the previously described protocol (15). Briefly, one llama was immunized six times

Fig. 6. Activity and dynamics of Vps34 complexes on membranes. (A) Vps30 BARA binds membranes, as shown by a decreased rate of HDX (blue). Regions showing an increase in HDX are depicted in orange. The membrane-protected region of Vps30 BARA, the activation loop, α 12 helix, and Vps15 N terminus (gray) suggests a plausible membrane-binding surface. (B) Activities of the Vps34 complexes on SUVs. Kinase-dead Vps15/Vps34 (Vps34-D731N) is used as a negative control. Error bars represent SD from the mean for triplicate measurements. (C) Complex II is active on GUVs, in sharp contrast to complex I, Vps15/Vps34, and the complex II aromatic finger mutant. Fifty GUVs were counted per condition. All GUVs showed PI3P production (red) for complex II, whereas none were positive for complex I, Vps15/Vps34, or the mutant. (D) A model for conformational changes on membranes. Regions showing increased HDX upon membrane binding (orange circles) and the dynamic C2/HELICAT linker helix (white circle) suggest pivot points. (E) A model of complex I on a highly curved membrane. The NTD of Atg14 and Vps38 C2 could contribute to anchoring the complexes at specific cellular sites.



with 100 μ g of cross-linked complex II. Peripheral blood lymphocytes were extracted 4 days after the final antigen boost. RNA was purified and converted into cDNA via reverse transcriptase polymerase chain reaction (PCR). The cDNA was cloned into phage display vector pMESy4 containing a C-terminal 6X His tag. For the phage selection, complex II (both cross-linked and native) was directly coated on the solid phase. Antigen-bound phages were recovered by proteolysis with trypsin. After selections, 53 clones were sequenced, of which 38 tested positive in an enzyme-linked immunosorbent assay (ELISA). Nanobody 8780, which was crystallized with complex II for structure determination, was selected using non-cross-linked material.

Expression and purification of nanobodies for crystallization

WK6 Su^- cells were transformed with the plasmids carrying VHH constructs genes from the selections described above. Cells were grown at 37°C to optical density at 600 nm = 0.7 and then induced with 1 M isopropyl- β -D-thiogalactopyranoside for 20 hours at 25°C. For the initial screening, 1 liter of cell culture was grown for each nanobody. For further characterization and crystallization trials, 6 liters of cell culture were grown. Nanobodies were purified from the cell periplasm. Cells from 1 liter of culture were resuspended on ice with 15 ml of TES buffer (200 mM Tris-HCl at pH 8, 0.5 mM EDTA, and 500 mM sucrose) and then rotated for 1 hour at 4°C. After incubation, 30 ml of 4 $\times$ diluted TES buffer (50 mM Tris-HCl at pH 8, 0.125 mM EDTA, and 125 mM sucrose) buffer was added to the cells, followed by further rotation for 45 min at 4°C. After ultracentrifugation, the supernatant was loaded onto Ni/NTA beads (Qiagen) equilibrated in wash buffer (50 mM phosphate at pH 7, 1 M NaCl) and washed extensively in the same buffer, followed by washing with a low-imidazole solution (50 mM phosphate at pH 7.4, 1 M NaCl, and 15 mM imidazole at pH 8). The protein was eluted with 200 mM imidazole at pH 8, 20 mM phosphate at pH 7.4, and 1 M NaCl and then concentrated in Amicon Ultra 5-kD cut-off concentrator before being loaded on a S75 10/30 gel filtration column (for small-scale 1-liter expression) or a S75 16/60 column (for large-scale 6-liter expression) equilibrated in 20 mM Tris-HCl at pH 7.5 and 150 mM NaCl.

Crystallization

Purified complex II was mixed with an excess of a purified nanobody, and the mixture was gel-filtered on a 16/60 Superdex 200 column. The relevant fractions were pooled; concentrated to between 6 and 7 mg/ml; and screened for crystallization in more than 1900 conditions using the LMB robotic crystallization setup (42), with 100 nl of protein and 100 nl of the reservoir solution in 96-well plates. Initial crystals of complex II were obtained using Crystal Screen 1 reagent 3 (Hampton Research), which contains 0.4 M monoammonium dihydrogen phosphate. The crystals were optimized in grids varying the concentration of

reagents; adjusting pH; and screening different salts, cryogenic protectants, detergents, and other additives. For data collection, crystals were produced in 96-well plates, using 200 nl of protein and 200 nl of the reservoir solution containing 0.35 M sodium acetate and 3% 1,5-diaminopentanedihydrochloride. Crystals were grown at 17°C and reached the maximum size after 5 to 7 days. Crystals were cryo-protected by adding to the drop a solution containing 0.35 M sodium acetate, 3% 1,5-diaminopentanedihydrochloride, and 25% ethylene glycol. Crystals were mounted in loops and flash-cooled in liquid nitrogen.

Structure solution

Diffraction data were collected at the European Synchrotron Radiation Facility on beamline ID29 and at the Diamond Light Source on beamlines I04 and I24. Native crystals of complex II bound to a nanobody diffracted to $\sim$ 4.4 Å resolution; some of these were soaked for 1 to 10 min in 2 mM tantalum bromide (Jena Biosciences PK-103) dissolved in the crystallization solution. We optimized data collection for tantalum-derivatized crystals by estimating the total dose absorbed using RADDOSE (43). Data sets for tantalum-soaked crystals were collected at the peak absorption edge and at the two different inflection points for this element (see table S1). Data were processed with XDS and scaled using XSCALE (44). Experimental phasing was carried out using either Sharp or Autosnap (45). The signal over noise for native crystals could be enhanced by combining seven isomorphous data sets together with XSCALE, which would allow us to adopt a 4.4 Å resolution cutoff.

A list of initial sites was obtained using SHELX C/D (46), enabling us to obtain a first experimental map in which previously solved models of the WD40 domain of Vps15, the BARA domain, coiled-coil domains of Vps30/38, and the HELCAT domain of Vps34 could be readily placed. At this stage, the electron density was clear enough to build most of the helices of the helical domain of Vps15. Missing parts of the complex, such as the kinase domain of VPS15 and the C2 domains of Vps34 and Vps38, were subsequently found using homemade scripts for serial molecular replacement with PHASER (47), with model libraries obtained from HHPRED secondary-structure profile matching hits (48) or BALBES (49). Improvement of the initial model was possible by successive rounds of model building and refinement, followed by density modification with SOLOMON (50) from the SHARP server interface using the updated model every time as a solvent mask to generate a better experimental map. Refinement was performed using tight secondary structure and Ramachandran restraints with PHENIX (51). Given the low resolution, no side chains were incorporated; however, the model contains the yeast sequence register.

HDX sample preparation: Dynamics and protein contacts

HDX-MS experiments were conducted to determine the complex dynamics and protein contacts within the complex. The D₂O used in all exper-

iments had $\geq$ 99.75% isotopic purity (Acros Organics). Protein samples [10 μ l of 2.5 μ M protein in dilution buffer (20 mM Tris at pH 8.0, 50 mM NaCl, and 2 mM TCEP)] were mixed rapidly with 35 μ l of D₂O buffer (20 mM Tris at pH 8.0, 50 mM NaCl, 2 mM TCEP, and 96.6% D₂O) to produce a sample with a final concentration of 75% D₂O. Four time points of exchange (3 s on ice; 3 s, 30 s, and 300 s at 23°C) were produced in triplicate. For each time point, the reaction was quenched by the addition of 20 μ l of quench buffer (8.4% formic acid and 5 M guanidine-HCl) and immediate freezing in liquid nitrogen. Samples were then stored at -80°C .

HDX sample preparation: Nanobody mapping

For nanobody-binding experiments, 10 μ l of either 2.5 μ M complex II or 10 μ l of a 2.5 μ M complex II/3 μ M nanobody solution were mixed rapidly with 40 μ l of D₂O buffer (producing a final concentration of 77% D₂O). Reactions were incubated for 3 s on ice before being quenched with 20 μ l of quench buffer and frozen in liquid nitrogen as described above.

HDX sample preparation: Membrane binding

Membrane-binding experiments were carried out using liposomes produced as described previously (28). Briefly, phospholipids were mixed in organic solution in the desired ratios {18% (w/v) liver phosphatidylinositol (Avanti 840042C), 45% brain phosphatidylserine (Avanti 840032C), 20% brain phosphatidylethanolamine (Avanti 840022C), and 17% brain phosphatidylcholine (Avanti 840053C) [grossly mimicking yeast plasma membrane, partly modified from (52)]}, desiccated, resuspended in lipid buffer (20 mM Tris at pH 8.0, 100 mM KCl, and 1 mM EGTA), sonicated, freeze-thawed 10 times, and then extruded 11 times through a 100-nm filter. For HDX-MS experiments, complex II (2.5 μ M final concentration) was incubated with either 1 mg/ml of liposomes or the equivalent volume of lipid buffer for 30 min before the addition of any deuterated buffer. HDX reactions were then conducted as described above, with 10 μ l of protein or protein-lipid solution being mixed with 40 μ l of D₂O buffer supplemented with either 1 mg/ml of liposomes or the equivalent volume of lipid buffer (76.6% D₂O). The final concentration of D₂O was 61%. Four incubations were conducted (3 s on ice; 3, 30, and 300 s at 23°C), and the samples were quenched and stored as noted above.

Measurement of deuterium incorporation

All samples were analyzed using the same chromatography system and mass spectrometry instruments. Protein samples were thawed and immediately injected into an ice-immersed ultra performance liquid chromatography (UPLC) system. The protein was initially passed through two immobilized pepsin columns (Applied Biosystems; poroszyme) at 150 μ l/min for 3 min to digest the protein, and the resulting deuterated peptides were collected on a VanGuard precolumn trap (Waters). The trap was then switched in-line to an Acquity 1.7- μ m particle, 100-mm-by-1-mm C18 UPLC column

(Waters), and the peptides were eluted from the column using a 20-min 5 to 45% gradient of buffer A (0.1% formic acid) and buffer B (100% acetonitrile), followed by a 4-min 76% buffer B wash. The mass of the eluted peptides was then determined using a Xevo quadrupole-time-of-flight mass spectrometer (Waters), acquiring for 30 min over mass/charge ratios of 350 to 1500, using an electrospray ionization source operated at a capillary temperature of 225°C and a spray voltage of 3.5 kV.

Peptide identification

The identification of nondeuterated peptides was determined by running tandem mass spectrometry (MS/MS) experiments with a nondeuterated sample digested as noted above but with a 60-min gradient over the reverse-phase column. This was repeated with a 20-min gradient, and identical peptides observed in both gradients were used to produce a linear extrapolation to correct for the retention times of peptides observed in the 60-min gradient. A tolerance of 15 parts per million was used in the MS observations, and an MS/MS tolerance of 0.2 daltons was also used. The resultant MS/MS data sets were analyzed using Mascot Distiller (Matrix Science). Peptides with a Mascot score lower than 10 were excluded from all further analysis. Centroid values were determined using HD-Examiner software (Sierra Analytics). Every nondeuterated peptide was validated manually by searching the protein sample's MS scan and checking for the correct charge state, the presence of any overlapping peptide envelopes, and a correct retention time (peptides failing any of these criteria were excluded from further analysis). Peptide coverage was as follows: Vps38, 51.9%; Vps34, 83.6%; Vps30, 82.9%; and Vps15, 36.8%.

Mass analysis of peptide centroids

Validated nondeuterated peptides were subsequently analyzed for their deuterium incorporation under various conditions. Due to overlap from the shift in isotopic envelope observed upon deuterium incorporation, additional peptides were discarded from the data set. All peptides were again checked for the correct charge state and retention time. All deuterium levels quoted in the figures and text are relative levels of deuterium, as no fully deuterated sample was obtained. A mathematical correction was applied using HD Examiner to compensate for the differences in the level of the deuterium in the various D₂O buffers. The average error was ≤ 0.5 daltons for any two replicates. Changes greater than 0.9 daltons for both observations were tested for significance with a Student's *t* test at the 95% confidence level. All results quoted in the figures and text are the greatest observed difference for that peptide at any single time point, unless otherwise explicitly stated.

CPY assay

Our CPY assay was modified from (17). Cells were grown at 30°C to mid-log phase [absorbance at 600 nm (A_{600}) = 0.8 to 1.0] in -URA medium, and an equivalent of five absorbance units of cells was collected and resuspended in 500 μ l of -URA medium with 50 mM KPO₄ at pH 5.7 and 0.5 mg/ml

bovine serum albumin (BSA) and incubated at 30°C for 1 hour. Cells and extracellular fractions were separated by centrifugation at 4000g for 1 min. Cellular fractions were subjected to the cell wall-loosening procedure (2 M LiAc on ice for 2 min, followed by 0.4 M NaOH on ice for 2 min), resuspended in a solution of 50 mM Tris at pH 8.0, 1% SDS, 8 M urea, 2 mM DTT, and 5 mM EDTA with glass beads, and disrupted at 3000 revolutions per minute (rpm) for 30 s with PowerLyzer (Mo Bio) at 4°C. One part of 4 $\times$ sample buffer was added to 3 parts of lysate and loaded on gel without boiling. Extracellular fractions were precipitated with final 10% trichloroacetic acid (from 100% stock) on ice for 30 min and centrifuged at 20,000g for 10 min. Pellets were washed two times with 90% cold acetone, air-dried, resuspended in 2 $\times$ sample buffer, and loaded on gel without boiling. CPY is synthesized as a premature form (p1), transported to the Golgi to be glycosylated (p2), and then delivered to the vacuole, where it is cleaved to a mature form (m). The p2 and m forms of CPY were detected both in cellular and medium fractions using anti-CPY (1/200; Invitrogen A6428). Vps38 proteins, which were all C-terminally tagged with 3 $\times$ Flag, were detected by anti-Flag-HRP (HRP, horseradish peroxidase) (1/1000; Sigma A8592). Both full-length Vps38 and Δ BARA2 show two protein bands. The bands with higher molecular weight correspond to the sizes of interest (asterisks in Fig. 5B). Pgl1 was detected by anti-Pgl1 (1/2000; Invitrogen 459250). Anti-mouse-HRP (1/2000; Sigma A9917) was used as a secondary antibody.

Protein A pull-down assay

Plasmids carrying VPS15-ZZ (pYO225) were cotransformed with an empty vector (pRS424), untagged VPS34 (pYO69), or HELCAT (pYO361) into a yeast strain (YOY193). Protein expression was induced by 2% galactose for 24 hours at 30°C in 500 ml of YM4 medium in the presence of 2% glycerol and 3% lactic acid. Cells were harvested in 50-ml tubes, then 5 ml of lysis buffer (50 mM Tris at pH 8.8, 300 mM NaCl, 1 mM DTT, 1% Triton, 0.5 mM PMSF, and an inhibitor tablet) and 3 g of glass beads were added. Cells were disrupted once with FastPrep-24 at 6.5 intensity for 45 s. Cell debris and glass beads were removed by centrifuging at 4000 rpm for 2 min. Supernatants were transferred to ultracentrifuge tubes and spun at 20,000g for 10 min. IgG beads (100 μ l of 50% slurry) were added to supernatants and rotated at 4°C for 2 hours. The protein-bound beads were transferred to Poly-Prep chromatography columns (Bio-Rad) and washed with 10 ml of wash buffer (50 mM Tris at pH 8.0, 300 mM NaCl, 1 mM DTT, 1% Triton X-100, and 0.5 mM PMSF) and 10 ml of wash 2 buffer (50 mM Tris at pH 8.0, 300 mM NaCl, and 1 mM DTT) by gravity flow. After draining the wash 2 buffer, IgG beads were resuspended in 500 μ l of wash 2 buffer. A fraction of lysate (0.04%) and beads (2%) were loaded on a gel.

Microscopy

Cells were grown to mid-log phase (A_{600} = 0.8 to 1.0) and then examined with an inverted micro-

scope (Nikon Eclipse TE2000) equipped with a charge-coupled device camera (CoolSNAP-HQ2, Roper Scientific, Tucson, AZ), a green fluorescent protein (GFP) filter (Chroma Technology, Rockingham, UT), and a differential interference contrast channel. Images were analyzed using ImageJ, and levels were adjusted with Adobe Photoshop.

Vps15/Vps34-Vps30/Vps38 reconstitution

A yeast plasmid carrying VPS30 and ZZ-VPS38 (pYO734) was transformed into the YOY193 strain. The transformed cells were grown overnight at 30°C in -URA medium and then shifted to 1 liter of induction medium (YM4, 2% glycerol, 3% lactic acid, 2% galactose, 5 μ M ZnCl₂, 55 mg/liter leucine, and 55 mg/liter tryptophan) at 30°C for 24 hours. Cells (~10 g) were obtained and split into two aliquots of 5 g in 50-ml tubes. Cells per tube were disrupted in 5 ml of lysis buffer (50 mM Tris at pH 8.8, 150 mM NaCl, 1 mM DTT, 1% Triton X-100, 0.5 mM PMSF, and an EDTA-free protease inhibitor tablet) and 3 g of glass beads using a FastPrep24 at 6.5 intensity for 45 s. Cell debris and glass beads were spun down at 2500g for 2 min. Supernatants were combined and spun at 20,000g for 10 min. IgG beads (100 μ l) were added to the supernatant and rotated at 4°C for 2 hours. For the IgG beads-only control, 50 μ l of IgG beads was added in 5 ml of lysis buffer and rotated as described above. The control and protein-bound IgG beads were transferred to Poly-Prep chromatography columns and washed with 10 ml of wash buffer (50 mM Tris at pH 8.0, 150 mM NaCl, 1 mM DTT, 1% Triton X-100, 5 mM ATP, 50 mM MgCl₂, and 2 mg/ml ribonuclease A) and 10 ml of reaction buffer (50 mM Tris at pH 8.0, 300 mM NaCl, and 1 mM DTT) by gravity flow. The protein-bound IgG beads were split into two halves, one for the control without Vps15/Vps34 and the other for the reconstitution with Vps15/Vps34. After draining the buffer, 225 μ l of reaction buffer was added to each column, and then 25 μ l of purified Vps15/Vps34 was added to a final concentration of 1 μ M. A binding reaction was performed on ice for 1 hour. Beads were washed once with 10 ml of wash 2 buffer (50 mM Tris at pH 8.0, 300 mM NaCl, 1 mM DTT, and 1% Triton X-100) and once with 10 ml of reaction buffer. 2.5% of total volume was loaded on a SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel for input and IgG bead fractions.

Lipid kinase assays

Lipid kinase assays were carried out using liposomes produced as described above. Phospholipids were mixed in organic solution in the desired ratios [18% (w/v) phosphatidylinositol, 10% phosphatidylserine, 17% phosphatidylethanolamine, and 55% phosphatidylcholine], desiccated in a nitrogen stream, and then resuspended in lipid buffer (25 mM HEPES at pH 8.0, 100 mM NaCl, and 1 mM EGTA) to make a 1-mg/ml stock. The stock was sonicated in a bath sonicator, freeze-thawed 10 times, and then extruded 11 times through a 100-nm filter.

Lipid kinase assays were performed using the Echelon K-3000 kit, a 96-well ELISA assay for detection of PI3P. Protein dilutions and lipid stocks were made in reaction buffer (25 mM Tris at pH 8.0, 150 mM NaCl, 2 mM EGTA, 0.1% CHAPS, 4 mM MnCl₂, and 2 mM TCEP). First, 5 µl of 20 nM enzyme was aliquoted into PCR tubes. The reaction was initiated by adding 5 µl of liposomes in reaction buffer with 100 µM ATP to give a final enzyme concentration of 10 nM and a final PI concentration of 50 µM. Control reactions without ATP were also set up for all proteins. The reaction was incubated for 1 hour at room temperature without agitation, stopped by addition of 16.6 mM EDTA, and diluted to 80 µl in the 2× PI3P detection buffer (K-3004). Next, 50 µl of this reaction solution was transferred to the detection plate (K-3001). PI3P was then detected on a plate reader according to the manufacturer's instructions. A kinase-dead Vps34 (D731N) in the Vps34/Vps15 dimer was used as a negative control. Error bars in Fig. 6B represent SD from the mean for triplicate measurements. The results shown are representative of at least three independent experiments.

Giant unilamellar vesicle (GUV) assay

Two concentric greased O-rings (16 and 18 mm in diameter) were placed on the metal side of an indium tin oxide-coated glass slide. An aliquot (10 µl) of the 1-mg/ml lipid mixture of 18% (w/v) liver phosphatidylinositol, 10% brain phosphatidylserine, 17% brain phosphatidylethanolamine, 55% brain phosphatidylcholine (catalog numbers as above), and 0.1% Lisamine Rhodamine-PE (Avanti 810850P) was pipetted into the center of the rings and dried in a vacuum for 1 hour. Swelling buffer (500 mM glucose, 220 µl) was added to each ring. The slide was then placed into the Vesicle Prep Pro GUV generator (Nanion) and covered with another indium tin oxide-coated glass slide. An ac field (1 V, 10 Hz) was applied to the slides for 4 hours at 60°C. The wells of the observation chamber (Lab Tek chamber 1, Borosilicate, 155411) were pre-equilibrated with 5 mg/ml of BSA for 4 hours and then washed with the observation buffer (50 mM HEPES at pH 8.0, 150 mM NaCl). GUVs (50 µl) were added to a solution with enzymes and a PI3P detection probe (p4OPX domain labeled with AlexaFluor647) to yield a 200-µl solution of observation buffer supplemented with 1 mM EGTA, 2 mM MnCl₂, 1 mM TCEP, and 50 µM ATP, with an enzyme concentration of 0.5 µM and a probe concentration of 7 µM. Confocal images were acquired using Zeiss LSM 780, with a ×63 (NA 1.4) oil objective lens.

Temperature-sensitivity experiment

The plasmids were transformed into SEY6210 (WT) or *vps34Δ*. After 2 days at 30°C on –URA plates, newly growing colonies were repatched onto new –URA plates and grown at 30° or 37°C for 2 days. Protein expression was also confirmed by Western blotting with anti-Flag-HRP.

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SUPPLEMENTARY MATERIALS

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Figs. S1 to S7

Tables S1 to S6

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Movie S1

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REPORTS

FRUSTRATED MAGNETISM

Hidden order in spin-liquid $\text{Gd}_3\text{Ga}_5\text{O}_{12}$ Joseph A. M. Paddison,^{1,2,3} Henrik Jacobsen,^{4,5} Oleg A. Petrenko,⁶ Maria Teresa Fernández-Díaz,⁷ Pascale P. Deen,^{4,5*} Andrew L. Goodwin^{1,3}

Frustrated magnetic materials are promising candidates for new states of matter because lattice geometry suppresses conventional magnetic dipole order, potentially allowing “hidden” order to emerge in its place. A model of a hidden-order state at the atomic scale is difficult to deduce because microscopic probes are not directly sensitive to hidden order. Here, we develop such a model of the spin-liquid state in the canonical frustrated magnet gadolinium gallium garnet ($\text{Gd}_3\text{Ga}_5\text{O}_{12}$). We show that this state exhibits a long-range hidden order in which multipoles are formed from 10-spin loops. The order is a consequence of the interplay between antiferromagnetic spin correlations and local magnetic anisotropy, which allows it to be indirectly observed in neutron-scattering experiments.

When a material undergoes a transition to an ordered state, its physical properties change. One of the greatest triumphs of 20th-century physics was to explain these macroscopic changes in terms of the microscopic ordering of atoms and magnetic dipole moments (spins). A canonical example is antiferromagnetic order, which remained hidden until the development of neutron-scattering experiments in the 1940s (*1*). Today, a similar situation exists in “hidden order” materials, where the order is of a type that cannot be directly probed by our current experimental techniques [see, e.g., (*2–4*)]. Exotic hidden-order states such as quantum spin nematics, chiral spin ices, and multipolar spin-orbital order have been theoretically proposed (*5–7*). Experimentally, the canonical example of a hidden-order state is the material URu_2Si_2 (*2, 8*).

Frustrated magnetic materials are promising candidates for the discovery of hidden-order states (*9*). In frustrated systems, the lattice geometry suppresses conventional (long-range) spin order and a “spin liquid” phase may exist instead (*10*). Crucially, the strong spin correlations in spin-liquid states can lead to spin clusters behaving as a single object. Even though individual spins remain in a liquid-like state (*11*), spin clusters may have properties that exhibit nondipolar long-range order. The experimental signature of a potential hidden-order state is an anomaly in thermodynamic measurements that is not accompanied by a transition to long-range or frozen spin order (indicated, e.g., by the development of magnetic Bragg peaks in neutron-diffraction experiments) (*2*). This anomaly may be either sharp, as in a conventional

second-order phase transition, or broad, as for the transition from the paramagnetic phase to the “Coulomb phase” in spin-ice materials (*12*).

The well-studied frustrated magnet $\text{Gd}_3\text{Ga}_5\text{O}_{12}$ [gadolinium gallium garnet (GGG)] exhibits thermodynamic anomalies of the second type. In GGG, a spin-liquid state is observed at temperatures above a spin-glass transition at $T_g \approx 0.14$ K (*13*). Evidence for a conventional spin liquid in GGG is the suppression of T_g to temperatures far below the antiferromagnetic interaction strength of ~ 2 K (*14–16*), the observation of liquid-like magnetic diffuse scattering in powder neutron-scattering measurements (*17*), and the persistence of strong spin fluctuations to low temperature (*18–20*). Yet, broad anomalies are present in both the nonlinear susceptibility χ_3 and the magnetic specific heat at around 0.5 K, which do not yet have a well-understood microscopic origin (*13, 21*).

The crystal structure of GGG is shown in Fig. 1A. The unit cell is cubic and contains 24 equivalent magnetic Gd^{3+} ions with spin quantum number $S = 7/2$. The Gd^{3+} ions form two interpenetrating networks of opposite chirality, each of which describes a three-dimensional arrangement of corner-sharing triangles known as hyperkagome. The local environment of the Gd^{3+} ions is shown in Fig. 1B, where local axes x , y , and z are defined by the three twofold rotational axes of point symmetry (table S1) (*22*). Of particular importance in the GGG structure are the 10-spin loops shown in blue in Fig. 1B, which are the shortest path connecting a Gd^{3+} ion back to itself beyond individual triangles. Each Gd^{3+} sits in the middle of exactly one loop; hence, loop and atom share the same point symmetry and the local axes defined for atoms apply equally to loops. In a simple model, which considers only nearest-neighbor antiferromagnetic interactions, collective rotations of the loop spins allow the system to move at no energy cost between its degenerate ground states (*23*). We therefore anticipate that the 10-spin loops should play a key role in any emergent behavior present in the spin-liquid state.

We performed single-crystal neutron-scattering measurements on GGG in order to probe the spin-

liquid state. These measurements are challenging because of the very large neutron absorption cross section of natural Gd, and a high-energy incident neutron beam was used to mitigate against this problem (*22*). The left-hand panels of Fig. 2A show single-crystal neutron-scattering data at $T = 0.175$ K. To model these highly structured magnetic diffuse-scattering data, we use the reverse Monte Carlo (RMC) method (*24, 25*) to perform quantitative refinement to magnetic diffuse-scattering data from (*17*), which were collected on a powder sample (fig. S1) (*22*). The RMC method has two important properties: It generates an atomic-scale model from which arbitrary (nondipolar) correlation functions can be calculated, and it does not introduce a predetermined set of magnetic interactions that could bias the results (*26*). Excellent agreement between the RMC fit and the powder data is achieved at $T = 0.175$ K (Fig. 2B). Calculations of the single-crystal scattering from the refined RMC model show close qualitative agreement with the experimental single-crystal data, which provides evidence for the validity of the RMC model (Fig. 2A, right panels). Consistent with Mössbauer measurements (*27*), we obtain a strongly anisotropic distribution of spin orientations with spins preferentially oriented within their local xy planes (Fig. 2B, inset, and fig. S2). Our RMC refinements do not determine the term in the Hamiltonian responsible for this xy anisotropy. However, additional simulations of a spin Hamiltonian for GGG (*15, 16*) showed that the magnetic dipolar interaction (which couples spin and space degrees of freedom) generates the same xy anisotropy [fig. S7) (*22*). The sensitivity of the powder-averaged data to local spin anisotropy was confirmed by showing that an isotropic model does not fit the data successfully (green line in Fig. 2B). Consistent with simulations (*28*), the radial spin correlation function $\langle \mathbf{S}(0) \cdot \mathbf{S}(r) \rangle$ reveals a rapid decay of spin correlations with an exponential correlation length $\xi = 4.951(2)$ Å (similar to the nearest-neighbor distance $r_{\text{nn}} = 3.78$ Å) and a nearest-neighbor spin correlation coefficient $\langle \mathbf{S}(0) \cdot \mathbf{S}(r_{\text{nn}}) \rangle$ close to -0.5 (Fig. 2C) (angle brackets denote a configurational average). The temperature dependence of ξ was obtained from fits to powder diffuse scattering data at higher temperatures (*22*) and shows a gradual decrease with increasing temperature (Fig. 2C, inset). Based on this analysis, we conclude that the spin anisotropy and short-range dipole order of our model are consistent with expectations.

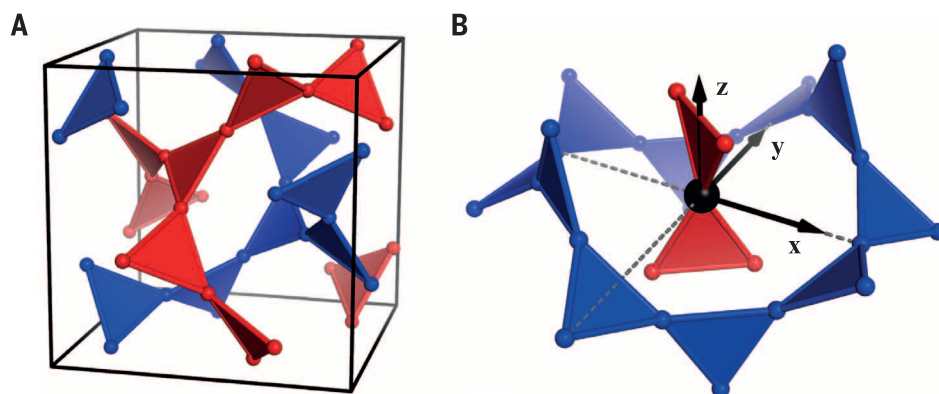
To look for exotic behavior beyond dipole order, we consider the 10-ion loops that are shown in Fig. 3A. Adjacent spins in a loop are nearest neighbors and are therefore antiferromagnetically correlated. Surprisingly, inspection of spin configurations suggested that strong antiferromagnetic alignment persists beyond nearest neighbors and throughout the loop, a result obscured by the radial average in Fig. 2C. To quantify this correlation, we calculate from our RMC configurations the average spin alignment axis of each loop (*22*),

$$\mathbf{L}(\mathbf{r}) = \frac{1}{10} \sum_n \cos(n\pi) \mathbf{S}_n(\mathbf{r}), \quad (1)$$

¹Department of Chemistry, University of Oxford, Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR, UK. ²ISIS Facility, Rutherford Appleton Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX, UK. ³School of Physics, Georgia Institute of Technology, Atlanta, GA 30332, USA. ⁴Nanoscience Center, Niels Bohr Institute, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark. ⁵European Spallation Source, Tunavägen 24, Lund, Sweden. ⁶Department of Physics, University of Warwick, Coventry, CV4 7AL, UK. ⁷Institut Max von Laue–Paul Langevin, F-38042 Grenoble 9, France.
*Corresponding author. E-mail: pascale.deen@esss.se (P.P.D.); andrew.goodwin@chem.ox.ac.uk (A.L.G.)

Fig. 1. Crystallographic properties of GGG.

(A) Crystal structure of GGG, showing only magnetic Gd^{3+} ions. The two interpenetrating networks of corner-sharing triangles are colored red and blue. (B) Local environment of Gd^{3+} ions. The local axes x , y , and z are defined by the three twofold axes of point symmetry of the Gd^{3+} site: $x \in (100)$ and $y, z \in \frac{1}{\sqrt{2}}(110)$, where z is chosen to pass through the centers of the two triangles that contain the Gd^{3+} ion. Each Gd^{3+} ion is located at the center of a loop of 10 Gd^{3+} ions from the other network, where the mean plane of the loop is perpendicular to the local z axis.



where $\mathbf{r}$ is the position of the center of the loop and $\mathbf{S}_n$ are unit-length spins within the loop ($n \in \{1, \dots, 10\}$). We refer to $\mathbf{L}(\mathbf{r})$ as the “10-spin director” to emphasize that $\mathbf{L}$ does not transform as a vector, because the twofold symmetry of the Gd^{3+} site requires that both $\mathbf{L}(\mathbf{r})$ and $-\mathbf{L}(\mathbf{r})$ identify the same alignment axis [our use of the director here mirrors the description of nematic liquid crystals (29)]. The average magnitude $\langle |\mathbf{L}(\mathbf{r})| \rangle = 0.49$ with standard deviation 0.18, which shows that antiferromagnetic correlation is strong within each loop and relatively consistent between different loops. The cyclic arrangement of 10 antiferromagnetic spins in a ring describes a multipole of order 6, which contains five nodal planes orthogonal to the plane of the loop and a single nodal plane coincident with it (Fig. 3A). Viewed in these terms, the normalized 10-spin director $\hat{\mathbf{L}} = \mathbf{L}/|\mathbf{L}|$ describes the multipole orientation associated with a given 10-ion loop. Figure 3B shows our key result: the distribution of normalized 10-spin directors is strongly peaked along the local z axis.

This result is notable because it shows that the fluctuations of the 10 spins in a loop select a single axis on average, so the rotational degree of freedom possessed by xy -like spins is lost in the loop directors. Hence, the 10-spin directors have no ground-state degrees of freedom and are ordered, with excitations normal to the local z axis (Fig. 3B). Moreover, because the 10-spin directors describe multipole orientations, the order is multipolar. The unit cell of this magnetic multipole crystal is shown in Fig. 3C. The multipole order preserves the symmetry of the crystal structure but is not required by this symmetry, which only constrains the distribution of 10-spin directors to preserve the three twofold rotation axes. Figure 3D shows the axial correlation function of the normalized 10-spin directors extracted from our RMC configurations (22),

$$g_L(r) = 2\langle \hat{\mathbf{L}}(0) \cdot \hat{\mathbf{L}}(r) \rangle - 1 \quad (2)$$

which is equal to -1 if, on average, loop directors separated by distance r are orthogonal to each other and to $+1$ if they are collinear. The correlation length of this function diverges within the statistical error of our refinements (22), as is to be expected from the axial distribution of 10-spin directors in Fig. 3B. Analogous to a noncollinear antiferromagnet, the noncollinearity of local z axes for different loops leads to $g_L(r)$

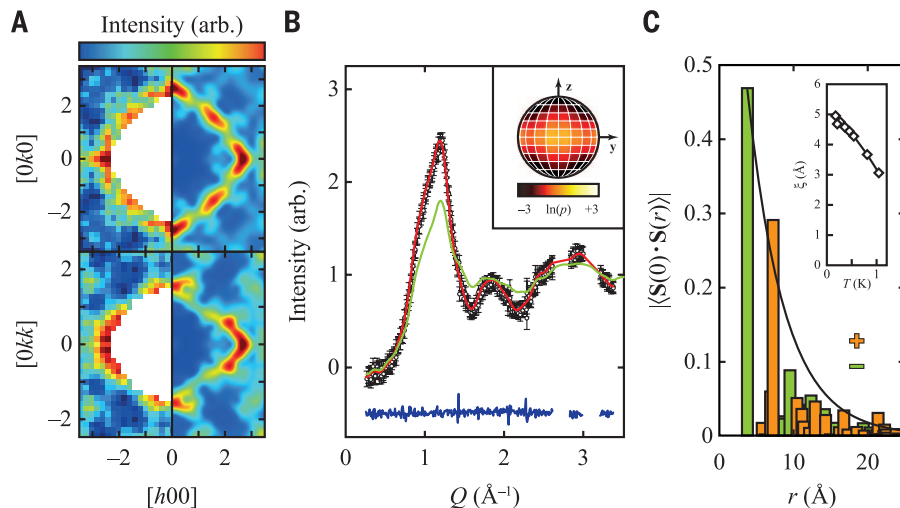


Fig. 2. Experimental data, fits, and calculations for GGG at $T = 0.175$ K. (A) Single-crystal magnetic diffuse scattering in two reciprocal-space planes. The upper image shows the $(hk0)$ plane, and the lower image shows the (hkk) plane. In each image, the left panel shows experimental data and the right panel shows the calculation from RMC refinements described in the text. Regions where there are no data are shown in white. (B) RMC fit to powder diffuse-scattering data [from (17)]. Data are shown as black circles, fit as a red line, and difference (fit – data) as a blue line. The scattering function calculated from the alternative isotropic model described in the text is shown as a green line. The inset shows a stereographic projection of the logarithmic probability distribution $\ln(\rho)$ of spin orientations [defined in (22)], revealing preferential spin alignment in the local xy plane (Fig. 1B). (C) Radial spin correlation function obtained from our RMC configurations. The bars show spin correlation values, and the solid black line shows a fit to an exponential envelope, $\pm \exp(-r/\xi)$, where $\xi = 4.951(2)$ Å. Correlations with positive (ferromagnetic) values are shown in orange, and correlation with negative (antiferromagnetic) values are shown in green. The inset shows the temperature dependence of ξ (the solid line is a guide to the eye). Error bars in (C) are smaller than the line thickness or symbol size in the plots.

taking several values but does not affect the divergence of the correlation length (fig. S3).

How is our analysis sensitive to nondipolar order, given that neutron scattering is a dipolar probe? To answer this question, we note that neutron scattering is directly sensitive to two quantities: the spin anisotropy and the spin-pair correlations (30). If an interplay of both quantities generates hidden order, neutron scattering may be indirectly sensitive to the hidden order itself. We performed Monte Carlo simulations, which show that this is the case for GGG: Long-range multipole order is present when both xy anisotropy and antiferromagnetic interactions are included but is absent for either (i) isotropic antiferromagnetic interactions or (ii) noninteracting spins with xy anisotropy [figs. S5 and S8] (22). Hence, hidden

order is not a consequence of either xy anisotropy or antiferromagnetic interactions alone.

The development of hidden order provides a plausible explanation for observed thermodynamic anomalies in the spin-liquid state of GGG. We show this by considering a simple model in which spins are constrained to lie in their local xy planes and are coupled by antiferromagnetic nearest-neighbor interactions. We do not claim that this xy model accurately represents the spin Hamiltonian of GGG but consider it instead because it is the simplest model showing the hidden-order state. From this model, we calculate two quantities: the magnetic specific heat C_{mag} and a limiting multipole correlation g_{max} , which we determine by fitting a straight line to $g_L(r)$ at the distances where it takes its maximum value

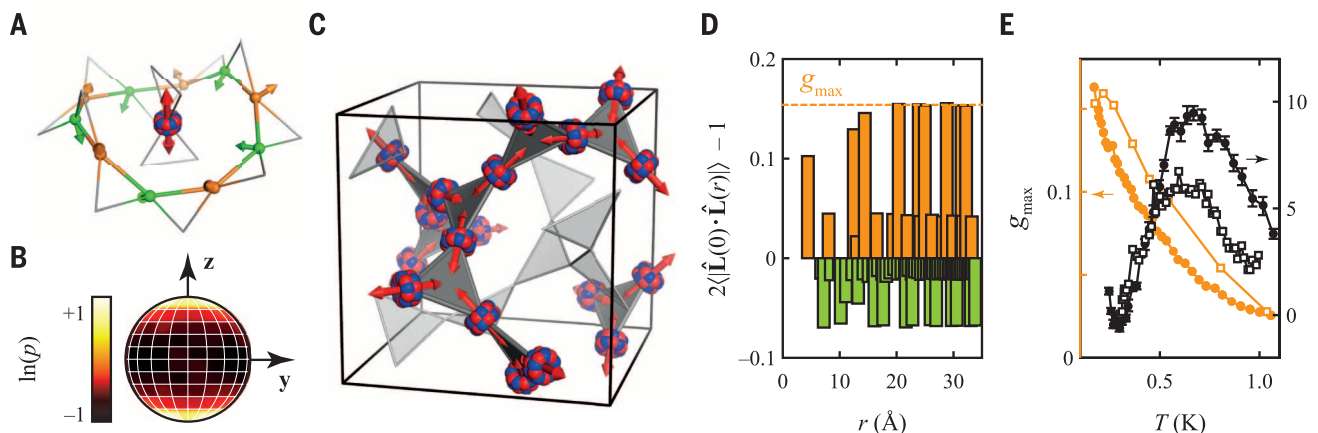


Fig. 3. Multipole order in GGG. (A) Relationship between representative spin orientations and 10-spin director for a 10-spin loop. Antiferromagnetically correlated spins are colored alternating green and orange as the loop is traversed. The multipole formed by the 10 spins is shown at the center of the loop, and the 10-spin director defined in the text is shown as a red double-headed arrow. (B) Stereographic projection showing that the logarithmic probability distribution function $\ln(p)$ [defined in (22)] of normalized 10-spin directors extracted from our RMC configurations (22) is strongly peaked along the local z axis [shown by the red double-headed arrow in (A)]. Fluctuations of the loop director normal to z are also apparent. (C) Crystal structure showing the unit cell of the magnetic multipole crystal. Multipoles and 10-spin directors are shown as in (A) and are illustrated for only one of the two networks for clarity. (D) Axial correlation func-

tion of normalized 10-spin directors calculated from our RMC configurations (22), showing the presence of long-range multipole order. Collinear correlations are shown as orange bars, and orthogonal correlations are shown as green bars. (E) Temperature evolution of the broad peak in the magnetic specific heat ΔC (black symbols) compared with temperature evolution of the limiting multipole correlation $g_{\max}$ (orange symbols). Open squares show experimental specific-heat data [from (13)] and $g_{\max}$ obtained from RMC refinement to experimental neutron-scattering data, whereas solid circles show specific heat and $g_{\max}$ calculated for a model with antiferromagnetic nearest-neighbor interactions and xy anisotropy as described in the text. The broad specific-heat peak has been isolated by fitting polynomial background functions to the experimental specific-heat data and model calculation (22).

for the multipole crystal—i.e., the distances separating loops that share the same local axes (Fig. 3D). Figure 3E compares the broad peak in the specific heat and $g_{\max}$ determined from the xy model with the experimental specific-heat peak and $g_{\max}$ determined from RMC refinement. We make three key observations. First, a broad specific-heat peak occurs in the xy model and the real material, which both show hidden order; moreover, this specific-heat peak is absent for models without hidden order (fig. S6) (22). Second, there is a large change in $g_{\max}$ over the temperature range where the specific-heat peak develops but only a much smaller change in the spin correlation length (Fig. 2C, inset). Third, the temperature evolution of the specific-heat peak correlates with the evolution of hidden order in a similar way for both xy model and experiment (Fig. 3E). These results suggest that the broad specific-heat anomaly is a signature of loss of entropy associated with the developing hidden order.

The hidden-order state that we propose for GGG does not break the crystal symmetry and is built from groups of spins that are individually fluctuating in space and time (18–20); for examples of how spin fluctuations occur consistently with multipolar order, see fig. S4 (22). It is also likely that the multipolar order will be apparent in larger clusters than the 10-spin loops. Compared with URu_2Si_2 (2), the lack of symmetry-breaking in GGG broadens the specific-heat anomaly observed as the hidden-order state develops, similar to the transition to the Coulomb phase in spin-ice materials (12). An interesting comparison can be drawn with frustrated spinels such as MgCr_2O_4 , in which hexagonal spin loops may form strongly ordered (“protected”) degrees

of freedom but adjacent loops are only weakly correlated (31, 32). The hidden order in GGG represents the opposite limit, in which the loops are long-range ordered, whereas individual spins show only short-range correlations. The hidden order in GGG therefore has properties fundamentally different from previous examples. Our results suggest that the atomic-scale refinement approach used here will prove valuable to unmask hidden-order states in other materials, such as chiral-spin liquids in frustrated magnetic materials (11) and spin nematics in high-temperature superconductors (33).

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6257/179/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S8
Table S1
Data Files S1 to S3
References (34–43)

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TOPOLOGICAL MATTER

Chiral solitons in a coupled double Peierls chain

Sangmo Cheon,¹ Tae-Hwan Kim,^{1,2} Sung-Hoon Lee,^{1*} Han Woong Yeom^{1,2*}

Chiral edge states are the hallmark of two- and three-dimensional topological materials, but their one-dimensional (1D) analog has not yet been found. We report that the 1D topological edge states, solitons, of the charge density wave system of indium atomic wires self-assembled on a silicon surface have chirality. The system is described by a coupled double Peierls-dimerized atomic chain, where the interchain coupling induces dynamical sublattice symmetry breaking. This changes its topological symmetry from $Z_2 \times Z_2$ to Z_4 and endows solitons with a chiral degree of freedom. Chiral solitons can produce quantized charge transport across the chain that is topologically protected and controllable by the soliton's chirality. Individual right- and left-chiral solitons in indium wires are directly identified by scanning tunneling microscopy.

Topological edge states that exist between two topologically distinct phases play a key role in quantum Hall insulators and topological insulators (1, 2). They are gapless and responsible for edge currents that are chiral in the sense that they propagate in one direction only (3, 4). The direction or chirality of the edge current in quantum Hall insulators depends on the sign of the difference of topological quantum numbers, called Chern numbers (5), of the two phases. The helical edge states of three-dimensional (3D) topological insulators are intrinsically chiral because they consist of two spin-dependent copies of chiral edge states for any transport axis at the surface (6, 7). Although the chirality is the essential feature of the edge states of 2D and 3D topological materials, there is no direct analog in 1D. Peierls-dimerized atomic chains such as polyacetylene (8) are prototypical 1D topological materials (9) and have topological edge states, known as Jackiw-Rebbi solitons (10), but they do not have chirality. In fact, “chiral” solitons are difficult to define because currents cannot be defined at the zero-dimensional edges (11). There have been theoretical proposals for nontrivial solitons, such as irrationally charged solitons (13–17), but no efforts have been made to endow solitons with chirality.

Here, we report that the 1D charge density wave (CDW) system of indium atomic wires self-assembled on a silicon surface (18–22) has chiral solitons, where the chirality is defined through dimensional extension by adiabatic phase evolution (23–25). The indium wire is described by a coupled double Peierls-dimerized atomic chain with four degenerate ground phases. The interchain coupling induces dynamical breaking of the sublattice symmetry and results in three types of topological edge states—right-chiral, left-chiral, and nonchiral solitons—each having distinct off-midgap

electronic states. The three types of solitons are identified with scanning tunneling microscopy and spectroscopy (STM/STS).

In our system, indium atoms deposited on the Si(111) surface at an elevated temperature self-organize into an ordered array of metallic wires (18). Each wire consists of two zigzag chains of indium atoms (Fig. 1A) (19) and possesses three

metallic bands crossing the Fermi level at room temperature (18), as shown in the band structure (Fig. 1B) obtained from density functional theory (DFT) calculations (20, 26). It undergoes a period-doubling CDW transition at around 125 K (18) to form the characteristic hexagon structure (20), mainly through the Peierls dimerization along the two outer indium rows (21). In this CDW state, one of the three surface bands (S_3) is lifted up above the Fermi level and the other two bands (S_1 and S_2) mix to open a band gap (22). The latter two bands play the major role in the CDW and soliton formation, as described below.

To study coupled Peierls chains, we generalized the tight-binding Su-Schrieffer-Heeger (SSH) Hamiltonian for a single Peierls chain (8, 26). In the single-chain SSH model of half-filled p orbitals, a spontaneous Peierls dimerization occurs with two possible dimer configurations (A and B phases in Fig. 1C) to gain electronic energy by opening a CDW gap (27). For a double chain with finite interchain hopping (insets of Fig. 1A), the half-filled band of each chain couples to form two split bands, S_1 and S_2 (Fig. 1D). The coupled chains also undergo a metal-insulator transition via the Peierls dimerization that occurs simultaneously in both chains. The ensuing structure allows for a mixing of S_1 and S_2 , resulting in a characteristic double Peierls gap. The band structures of this double-chain model (Fig. 1D)

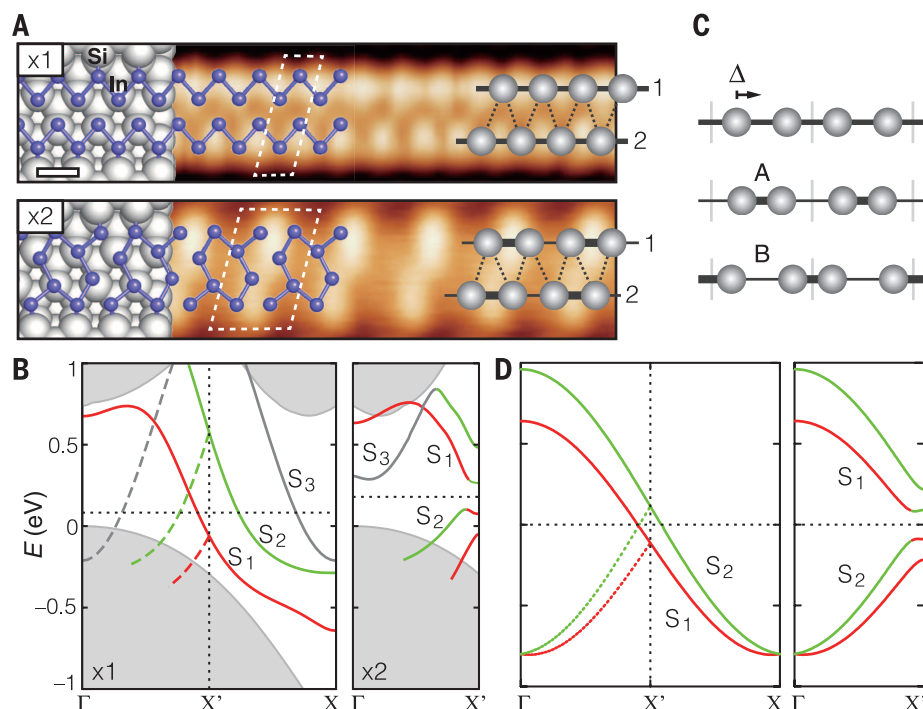


Fig. 1. A coupled double Peierls chain in indium atomic wires. (A) STM images and the corresponding atomic structures of indium wires self-assembled on the Si(111) surface before ($\times 1$) and after ($\times 2$) the period-doubling CDW-driven structural transition. Gray spheres and indigo balls represent silicon and indium atoms, respectively. Unit cells are marked by dashed lines. The size of the scale bar is 3.84 Å. Bright (dark) regions in the constant-current STM images correspond to higher (lower) tip heights with the height corrugation of ~ 1 Å. Effective models of the coupled double chains are overlaid on the right. (B) Band structures of indium wires before and after the structural transition obtained by DFT calculations (26). Dashed lines indicate band folding by the $\times 2$ CDW transition. Horizontal dotted lines indicate the Fermi level. (C) Schematic of two dimerized phases, A and B, of a single Peierls chain. (D) Band structures of the double Peierls chain before and after the dimerization obtained by tight-binding calculations (26).

¹Center for Artificial Low Dimensional Electronic Systems, Institute for Basic Science (IBS), Pohang 790-784, Korea.

²Department of Physics, Pohang University of Science and Technology (POSTECH), Pohang 790-784, Korea.

*Corresponding author. E-mail: shlee@ibs.re.kr (S.H.L.); yeom@postech.ac.kr (H.W.Y.)

reproduce the two characteristic bands of indium wires (Fig. 1B) for both metallic and CDW phases. Here, the particular type of the interchain coupling, the zigzag geometry, is crucial to reproduce these electronic structures (fig. S1).

As a consequence of the dimerization on both chains, the double chain has four degenerate ground phases (Fig. 2A), which are denoted as AA, BA, BB, and AB. This double-chain model describes correctly the fourfold degeneracy of the hexagon structure of the indium wire, which was well established experimentally (20, 21). Without the interchain coupling, the four ground states are simply a trivial product of the degeneracy of each chain, belonging to the $Z_2 \times Z_2$ topological class. However, the zigzag interchain coupling imposes finite but opposite onsite potentials for two sublattices in each chain to break the sub-

lattice symmetry of each chain (26). As a result, the coupled system exhibits a fourfold rotational symmetry (C_4) in the (Δ_1, Δ_2) space (Fig. 2B), where Δ_1 and Δ_2 are the dimerization displacement of each chain (26).

Electronic wave functions reveal interesting topological band structures of the system. For a single Peierls chain in the A phase, the valence and conduction band edge states across the CDW gap are the bonding and antibonding states of the sublattice orbitals, which can be represented as $|S_x^- \rangle = \begin{pmatrix} 1 \\ -1 \end{pmatrix}$ and $|S_x^+ \rangle = \begin{pmatrix} 1 \\ 1 \end{pmatrix}$, respectively (fig. S2) (9, 26). In the Bloch sphere, these states correspond to the sublattice pseudospin vectors pointing to $-\hat{x}$ and $+\hat{x}$, respectively. In the B phase, the direction of the pseudospin vectors is inverted, corresponding to a band inversion, an

essential feature of a topological material. For a double Peierls chain in the AA phase, the pseudospin vectors of band edge states have finite S_z components, as well as the S_x components (Fig. 2C), owing to the sublattice symmetry breaking. Importantly, if the phase changes from AA to BA—i.e., the sign of S_x of chain 1 is inverted—then chain 2 exhibits a sign inversion in S_z by the interchain coupling. Thus, at the domain boundary between the AA and BA phases, band inversion occurs in both chains. Similar changes occur among all four degenerate phases (fig. S3). This result and the C_4 symmetry indicate that the double Peierls chain has a unique topological band structure with the cyclic Z_4 symmetry.

Whereas the two degenerate phases of a single Peierls chain result in a pair of soliton and anti-soliton, the four degenerate phases dictate 12

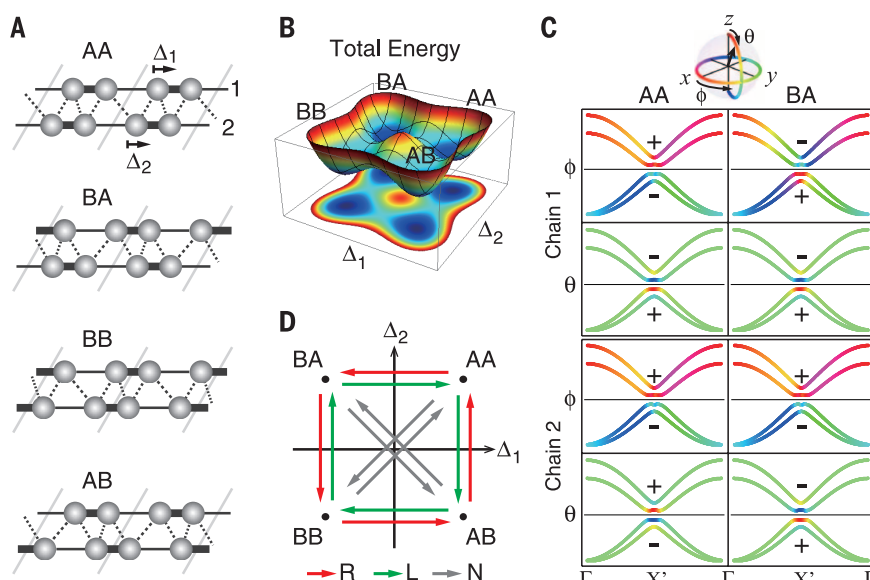


Fig. 2. Four degenerate ground phases. (A) Atomic configurations of four equivalent ground phases—AA, BA, BB, and AB—of a coupled double Peierls chain. (B) Total energy surface in the phase space of dimerization displacements of each chain (Δ_1, Δ_2). (C) Sublattice pseudospin of each Bloch state for each chain, color-coded in the band structure, for the AA and BA phases. The plot labeled by ϕ describes the azimuthal component of the pseudospin vector—i.e., the direction in the S_x - S_y plane. The sign of S_x is labeled at band edges. The plot labeled by θ describes the polar component of the pseudospin vector—i.e., the S_z value. The sign of S_z is labeled at band edges. (D) Diagram of topological solitons for the double chain. Owing to the fourfold rotational symmetry, 12 solitons are grouped into three types: right-chiral (R), left-chiral (L), and nonchiral (N) solitons.

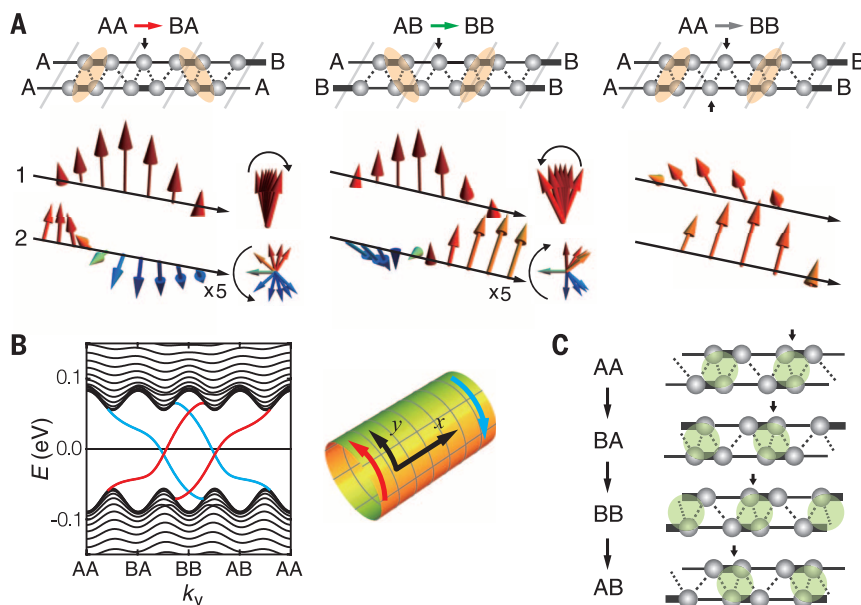


Fig. 3. Chiral solitons. (A) Schematics of solitons and wave functions of primary soliton states for each type of soliton. Unpaired kink sites are marked by black arrows. The ovals are guides for comparison with the STM images in Fig. 4B. Soliton wave functions are represented by sublattice pseudospin vectors for every pair of atoms. (B) Band structure of a double Peierls chain of a finite length (left), where the momentum (k_y) is taken as a parameter for the phase evolution of AA $\rightarrow$ BA $\rightarrow \dots \rightarrow$ AA, and the illustration of corresponding chiral edge currents in the 2D real space of a cylindrical geometry (right), where the y coordinate corresponds to the frequency domain of the phase evolution. The red and cyan bands correspond to localized edge states at left and right edges, respectively. (C) Center of the bonding charges of each phase. The phase evolution of AA $\rightarrow$ BA $\rightarrow \dots \rightarrow$ AA leads to charge pumping from the right edge to the left.

solitons among them (Fig. 2D). They can be categorized into three types owing to the C_4 symmetry: right-chiral (R), left-chiral (L), and nonchiral (N) solitons. The chiral nature of the soliton will be elaborated below. Figure 3A shows the schematic structures of three representative solitons and their soliton wave functions in a pseudospin description: $AA \rightarrow BA$, $AB \rightarrow BB$, and $AA \rightarrow BB$. The unpaired kink site in the right-chiral soliton has a different interchain coupling and thus has a different sublattice potential from that in the left-chiral soliton, and the nonchiral soliton has two unpaired kink sites, one at each chain. The different sublattice potentials are reflected in the wave functions: The sublattice pseudospins along the chain helically rotate in opposite directions in the right-chiral and left-chiral solitons, whereas their direction is fixed for the nonchiral soliton.

The three types of solitons have distinct electronic spectra (Fig. 4A). All solitons have two states within the gap but at different energies: The right-chiral (left-chiral) soliton has both states below (above) the midgap, but the nonchiral one has one below and the other above the midgap. For the nonchiral soliton, the two gap states are simply the bonding and antibonding states of the two soliton states of each chain. For the chiral solitons, consider the right-chiral $AA \rightarrow BA$ soliton as an example. When the interchain coupling δ is zero, it has a soliton state at chain 1 at zero energy, with a charge of e when occupied (adding contributions from both spins) (8, 10). As δ increases, the

energy level is lowered by an induced sublattice potential and the charge state decreases from e , similarly to the Rice-Mele model (13, 14). On the other hand, the induced sublattice potential of chain 2 exhibits a sign inversion near the kink of chain 1 and produces another soliton state emerging from the valence band edge with a small irrational charge (26). Interestingly, the total charge of the two soliton states is conserved to be e , regardless of the size of δ , owing to the C_4 symmetry. Thus, when the Fermi level is at the midgap, the right-chiral soliton is negatively charged by e , and similarly the left-chiral soliton is positively charged, whereas the nonchiral soliton is neutral. This integer charge state has an important consequence in the charge-transport behavior, as discussed below.

The three types of solitons are indeed observed in indium wires. Figure 4B displays STM images of indium wires with different solitons. The left and right sides of these wire segments correspond to different ground phases with kink sites at their interfaces. The CDW crests are translated or flipped across the kink sites. The nonchiral soliton was analyzed previously (21) and can largely be understood by two normal solitons at each chain. However, for the chiral solitons, the detailed STM line profiles indicate a distinct structural property (Fig. 4C). Whereas the chain with a kink shows a typical soliton profile of a hyperbolic tangent function, the other single-phase chain exhibits a small double-dip amplitude modulation. These struc-

tural features are well reproduced by the calculated atomic displacement fields of the double Peierls chain model (fig. S8). Beyond the structures, the STS revealed distinct local density of states (LDOS) for three different solitons (Fig. 4A). The right-chiral (left-chiral) soliton sites show strong LDOS enhancements near the bottom (top) of the band gap but the nonchiral solitons near both the bottom and top of the gap. This agrees reasonably well with the model calculations, considering the limited resolution of the STS data.

The concept of the “chiral” soliton that we propose is inherited from the chiral edge states of 2D quantum Hall systems. Consider a cyclic phase evolution of the 1D system along $AA \rightarrow BA \rightarrow BB \rightarrow AA$. This evolution corresponds to transporting four right-chiral solitons sequentially through the chain from the right edge to the left. It is topologically nontrivial in the sense that it encircles the origin in the (Δ_1, Δ_2) space (Fig. 2D), where the origin is singular with a zero band gap. An effective 2D Hamiltonian of the system, with the second dimension in the momentum space being the cyclic evolution (25), represents a Chern insulator with a Chern number of -2 (26), corresponding to a double sheet of the Haldane’s honeycomb lattice model for the quantum Hall effect (4). Figure 3B shows the band structure of a finite-length chain under the cyclic evolution. There are four topologically protected edge states crossing the zero energy, two localized at the left edge and the others at the right

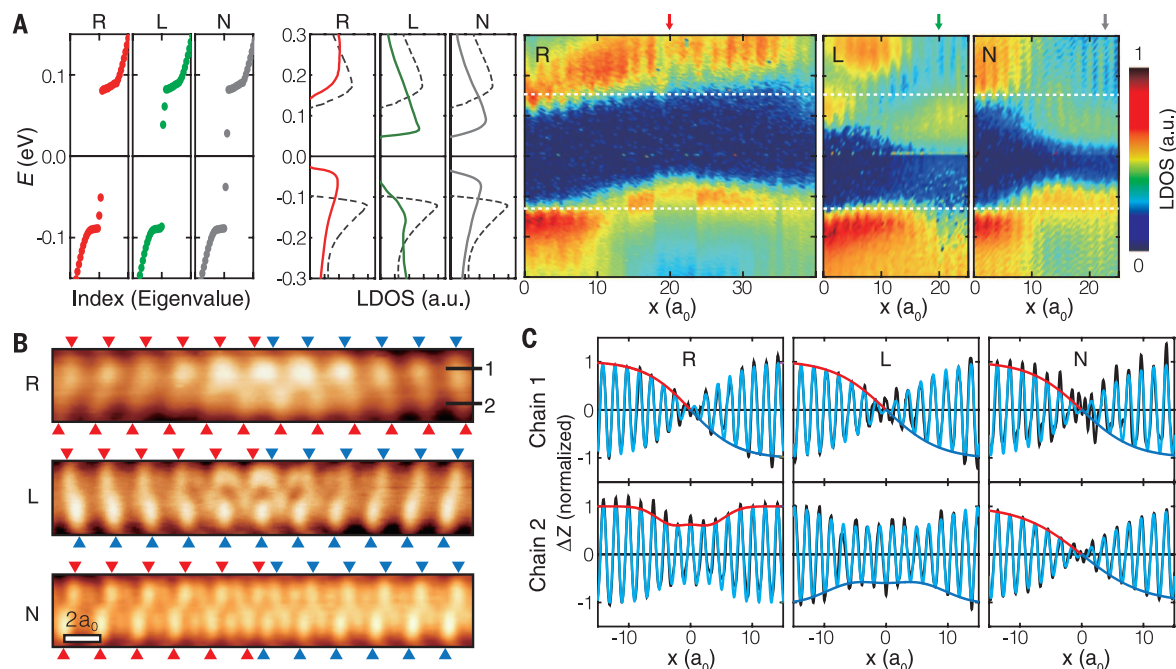


Fig. 4. Experimental observation. (A) (Left) Calculated eigenvalue spectra of each type of soliton. All solitons have two in-gap states. (Center) LDOS obtained by STS measurements at the center of each soliton site (solid lines), in comparison with that at a CDW ground state (dashed lines). (Right) Position-dependent LDOS data. The blue region of low LDOS represents the energy band gap. The center position of each soliton is marked by an arrow at the top. a_0 is the “ $\times 1$ ” period along the chain (3.84 Å). (B) STM images of each type of soliton. The red and blue triangles are guides

for local phases at each chain, A and B, respectively. The short lines on the right indicate where the line profiles of Fig. 4C are taken. (C) STM line profiles along the upper (chain 1) and lower (chain 2) parts of an indium wire. The STM line profiles (black) were band-pass filtered and corrected to include the effect of lattice modulation and Friedel oscillation (fig. S7). The cyan lines are fitted soliton profiles convoluted with the CDW modulation. The red and blue lines are fitted soliton profiles, with the color indicating the local phase.

edge in the 2D real space of a cylindrical geometry. From the viewpoint of the 1D chain, the band structure implies that two electrons per spin are pumped from the right edge to the left by the cyclic evolution, and the charge pumping is topologically protected. For the reverse evolution by transporting left-chiral solitons, the edge currents in the 2D system flow in the opposite direction, and so does the charge pumping in the 1D chain. In contrast, for the nonchiral evolution of $AA \rightarrow BB \rightarrow AA$, which do not encircle the origin in the phase space, the edge states are not chiral, and there is no charge pumping (26).

The quantized charge pumping by chiral solitons can be understood physically by considering electric polarization. Figure 3C depicts the center of the bonding charges, called the Wannier center (28), for the four ground phases. For the right-chiral evolution of $AA \rightarrow BA$, the charge center moves to the left, resulting in the increase of electric polarization by $|e|$ (26). Thus, it produces charge pumping of one electron from the right edge to the left. For the nonchiral evolution of $AA \rightarrow BB$, the charge center moves by half of the unit cell, and the bonding charges split into both the left and right directions, producing no change in polarization and no charge pumping.

For a 1D system to have a chiral soliton, it should have more than two degenerate ground phases, and a set of its solitons should form a topologically nontrivial closed path in the phase space. Solitons in systems with two degenerate ground phases (8, 14) are not chiral, regardless of whether the sublattice symmetry is broken or not (29), and thus for those systems charge pumping is not possible unless there are external time-varying potentials (23, 24, 30). In our system with four ground phases, charge pumping is allowed by transporting chiral solitons. The pumping of four electrons by one-cycle evolution is topologically protected—i.e., secure from defects or perturbations that do not close the band gap. Furthermore, because of the C_4 symmetry among the ground phases, each soliton delivers just one charge across the chain. This property can be exploited for future information storage devices where each bit of information is written by a single electron (37).

Our work suggests further studies of the 1D counterparts of other interesting topological systems in higher dimensions, such as the 1D realization of the quantum spin Hall system.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S9
References (32–36)

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SURFACE CHEMISTRY

Finding optimal surface sites on heterogeneous catalysts by counting nearest neighbors

Federico Calle-Vallejo,^{1,2,*} Jakub Tymoczko,^{3,4,*} Viktor Colic,^{3,5} Quang Huy Vu,⁶ Marcus D. Pohl,⁵ Karina Morgenstern,⁶ David Loffreda,¹ Philippe Sautet,^{1,†} Wolfgang Schuhmann,^{3,4} Aliaksandr S. Bandarenka^{3,5,7,†}

A good heterogeneous catalyst for a given chemical reaction very often has only one specific type of surface site that is catalytically active. Widespread methodologies such as Sabatier-type activity plots determine optimal adsorption energies to maximize catalytic activity, but these are difficult to use as guidelines to devise new catalysts. We introduce “coordination-activity plots” that predict the geometric structure of optimal active sites. The method is illustrated on the oxygen reduction reaction catalyzed by platinum. Sites with the same number of first-nearest neighbors as (111) terraces but with an increased number of second-nearest neighbors are predicted to have superior catalytic activity. We used this rationale to create highly active sites on platinum (111), without alloying and using three different affordable experimental methods.

Slight changes in the surface structure of catalytic materials can have large impacts on the products and energetics of chemical reactions (1, 2). However, not all sites at catalytic surfaces have the same activity

or selectivity, which gives rise to the concept of structure sensitivity (3–5). Generally, identification of active sites is a challenging task that requires the combination of several approaches (6, 7). A rational way of designing catalysts should first identify the optimal active sites and then engineer surfaces where the presence of such sites is maximized. Modern computational screening techniques can provide the atomic-scale insight needed to elaborate simple catalyst design rules (2, 8). Such rules are the starting point to engineer target active sites on catalytic surfaces (9–12). The connection between these two steps must be straightforward and clear, which is not trivial in practice. The difficulties originate from an important detail: Existing computational techniques outline optimal energetic properties, which can be met by countless materials. Therefore, it is desirable to create procedures that generate more precise design rules.

Consider the specific case of the oxygen reduction reaction (ORR), $O_2 + 4(H^+ + e^-) \rightarrow 2H_2O$, a

¹Université de Lyon, CNRS, École Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, Laboratoire de Chimie, 46 Allée d'Italie, 69364 Lyon Cedex-07, France. ²Leiden Institute of Chemistry, Leiden University, Post Office Box 9502, 2300 RA Leiden, Netherlands. ³Center for Electrochemical Sciences, Ruhr-Universität Bochum, Universitätsstrasse 150, 44780 Bochum, Germany. ⁴Analytical Chemistry, Ruhr-Universität Bochum, Universitätsstrasse 150, 44780 Bochum, Germany. ⁵Energy Conversion and Storage, Physik-Department, Technische Universität München, James-Frank-Straße 1, 85748 Garching, Germany. ⁶Physical Chemistry I, Ruhr-Universität Bochum, Universitätsstrasse 150, 44780 Bochum, Germany. ⁷Nanosystems Initiative Munich, Schellingstraße 4, 80799 Munich, Germany.

*These authors contributed equally to this work.

†Corresponding author. E-mail: bandarenka@ph.tum.de (A.S.B.); philippe.sautet@ens-lyon.fr (P.S.); f.calle.vallejo@chem.leidenuniv.nl (F.C.-V.)

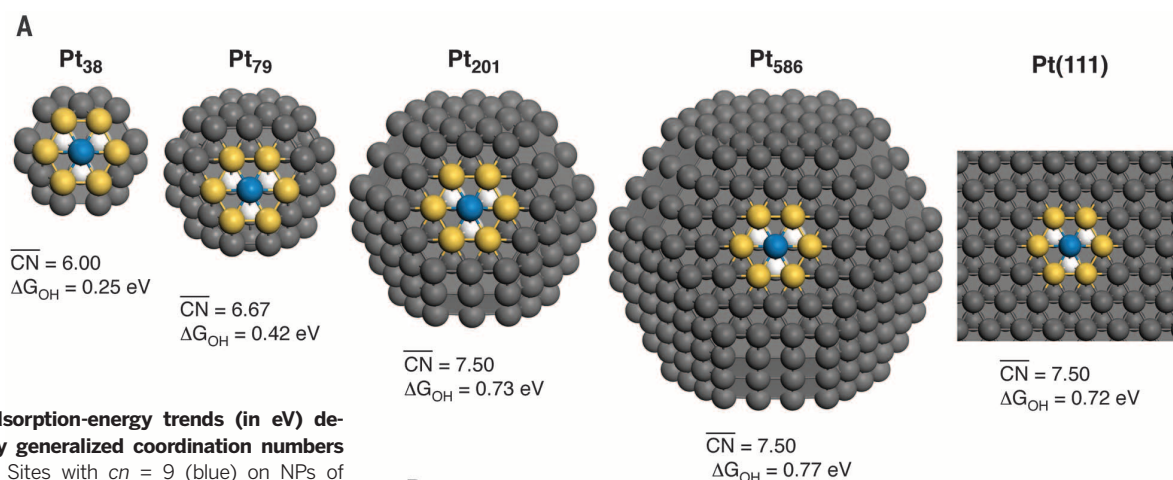


Fig. 1. Adsorption-energy trends (in eV) described by generalized coordination numbers ($\overline{CN}$). (A) Sites with $cn = 9$ (blue) on NPs of various sizes and on the extended (111) surface. The six surface nearest neighbors (yellow) and the three in the subsurface (white) are marked. Despite the identical coordination ($cn = 9$), ΔG_{OH} can differ by ~ 0.5 eV. The differences are due to the second-nearest neighbors. (B) Trends in ΔG_{OH} and ΔG_{OOH} described by $\overline{CN}$, including extended surfaces (brown) and truncated octahedron (●), cuboctahedron (■), and tetrahedron (▲) NPs: Pt₅₈₆ (yellow), Pt₂₀₁ (blue), Pt₁₄₇ (gray), Pt₇₉ (magenta), Pt₆₈ (orange), and Pt₃₈ (green). The reactions used to calculate the adsorption energies appear as insets. Least-squares fits are provided together with mean and maximum absolute errors (MAE and MAX).

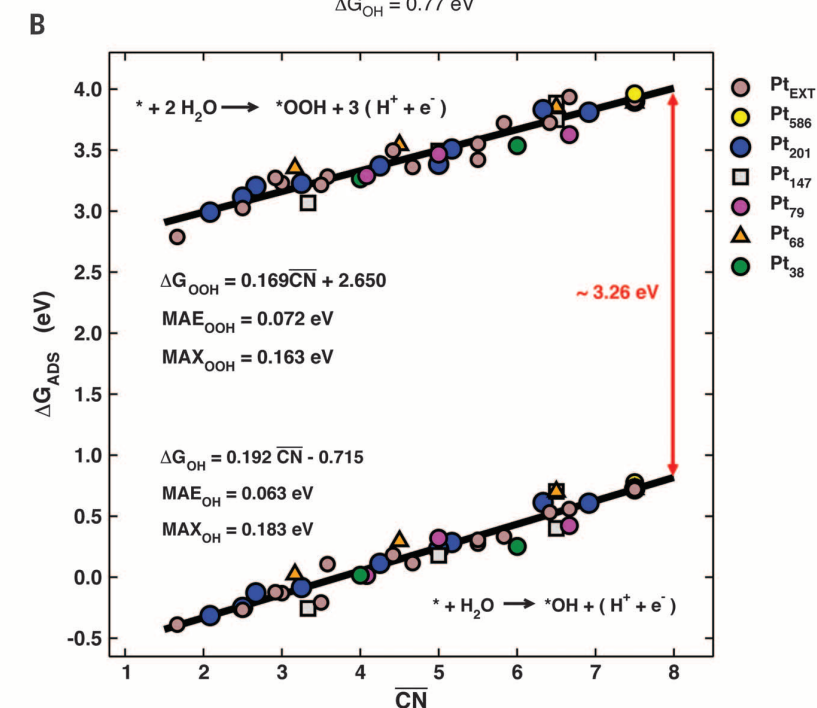


Fig. 2. Coordination-activity plot. (A) Potentials for the two limiting steps on extended surfaces and NPs. Points B and C (in light blue) are given for two cavities on Pt(111). The potential-determining step on the left (low coordination – strong binding) and right (high coordination – weak binding) sides of the volcano are indicated. Theoretical overpotentials (η_{ORR}) are the vertical difference between the points and the equilibrium potential (red dashed line). Optimal catalysts have $\overline{CN} \approx 8.3$ and $*OH$ adsorption energies ~ 0.15 eV weaker than Pt(111) (area in gray). (B) Top view of a six-atom cavity on Pt(111) with $\overline{CN} = (6 \times 10 + 3 \times 12)/12 = 8.00$. (C) Five-atom cavity on Pt(111) with $\overline{CN} = (4 \times 10 + 2 \times 11 + 3 \times 12)/12 = 8.17$.

key reaction for proton exchange membrane fuel cells that is normally catalyzed by Pt, which is scarce and expensive. Although many challenges remain (13), rational catalyst design has opened new avenues for the ORR through volcano-type activity plots (8, 14–16). These plots are based on the Sabatier principle, which states that good catalysts balance the strength of adsorption and desorption of key reaction intermediates. Volcano

plots typically correlate surface adsorption energies with estimates of the catalytic activity of materials. By means of density functional theory (DFT) calculations, they predict that optimal ORR catalysts must bind hydroxyl species (*OH) ~0.1 eV (2.3 kcal/mol) more weakly than Pt(111) (8, 15). Several studies have used this criterion to find alloy catalysts with high ORR activities (8, 12, 15, 17). However, this condition can be met by numer-

ous materials, including metals and alloys (15), oxides (18), functionalized graphitic materials, and porphyrins (19, 20). Therefore, screening routines are used to select suitable candidates from large databases in which the composition, structure, and adsorption energies of materials are known beforehand. Creating such databases demands considerable time and computational expenses.

We introduce here “coordination-activity plots,” which outline the geometric structure of optimal active sites. The method is illustrated on the ORR, for which we devise Pt catalysts that are 3.5 times more active than Pt(111).

Usually, trends in adsorption energies for small species on extended surfaces of a given transition metal are well described by the coordination number (cn) of the surface sites (21, 22). However, cn loses its accuracy when nanoparticles (NPs, the typical form of metals on high-surface area catalysts) are considered because of “finite-size effects” (23, 24), so more sophisticated descriptors are needed (25). For example, in Fig. 1A, all sites in blue have nine nearest neighbors, that is $cn = 9$, but the adsorption energies of *OH calculated with DFT [see Fig. S21 and tables S2 and S3 in the supplementary materials (26)] can differ by more than 0.5 eV (11.5 kcal/mol). A simple strategy to allow for direct comparison of NPs and extended surfaces is the use of “generalized” coordination numbers ($\overline{CN}$) (27), which introduce a weight to each first-nearest neighbor atom j , corresponding to its own coordination number [$cn(j)$]. The formula used to estimate $\overline{CN}$ for a site i is (27)

$$\overline{CN}(i) = \sum_{j=1}^{n_i} \frac{cn(j)}{cn_{\max}} \quad (1)$$

The sum includes all of the first-nearest neighbors, and the division by the maximum number of first-nearest neighbors in the bulk ($cn_{\max}$) ensures that $\overline{CN}$ spans the range between 0 and 12 in face-centered cubic metals, similarly to conventional cn . For instance, the blue site on Pt₃₈ in Fig. 1A has six neighbors with $cn = 6$ (yellow) and three with $cn = 12$ (white), so $\overline{CN} = (6 \times 6 + 3 \times 12)/12 = 6.00$ [section S3.1 in (26) shows how to compute $\overline{CN}$ for all sites under study]. This simple extension explains the variations in Fig. 1A and results in the linear relation in Fig. 1B, where the trends in adsorption energies of *OH and *OOH, ΔG_{OH} and ΔG_{OOH} , on all sites on Pt NPs of various sizes (0.7 to 2.6 nm) and shapes (truncated octahedron, cuboctahedron, tetrahedron) and extended surfaces are presented; data are reported for terraces, edges, corners, steps, kinks, and metal adatoms [see tables S2 and S3 in (26)]. Figure S21 shows that $\overline{CN}$ describes adsorption-energy trends more accurately than cn . Additionally, because $\overline{CN}$ is arithmetical, its assessment does not require DFT calculations.

Following the ORR model in (16), the two potential-determining steps are [see Fig. 2 and section S1 in (26)] (i) the first proton-electron transfer, in which O₂ is transformed into *OOH; or (ii) the last proton-electron transfer, in which

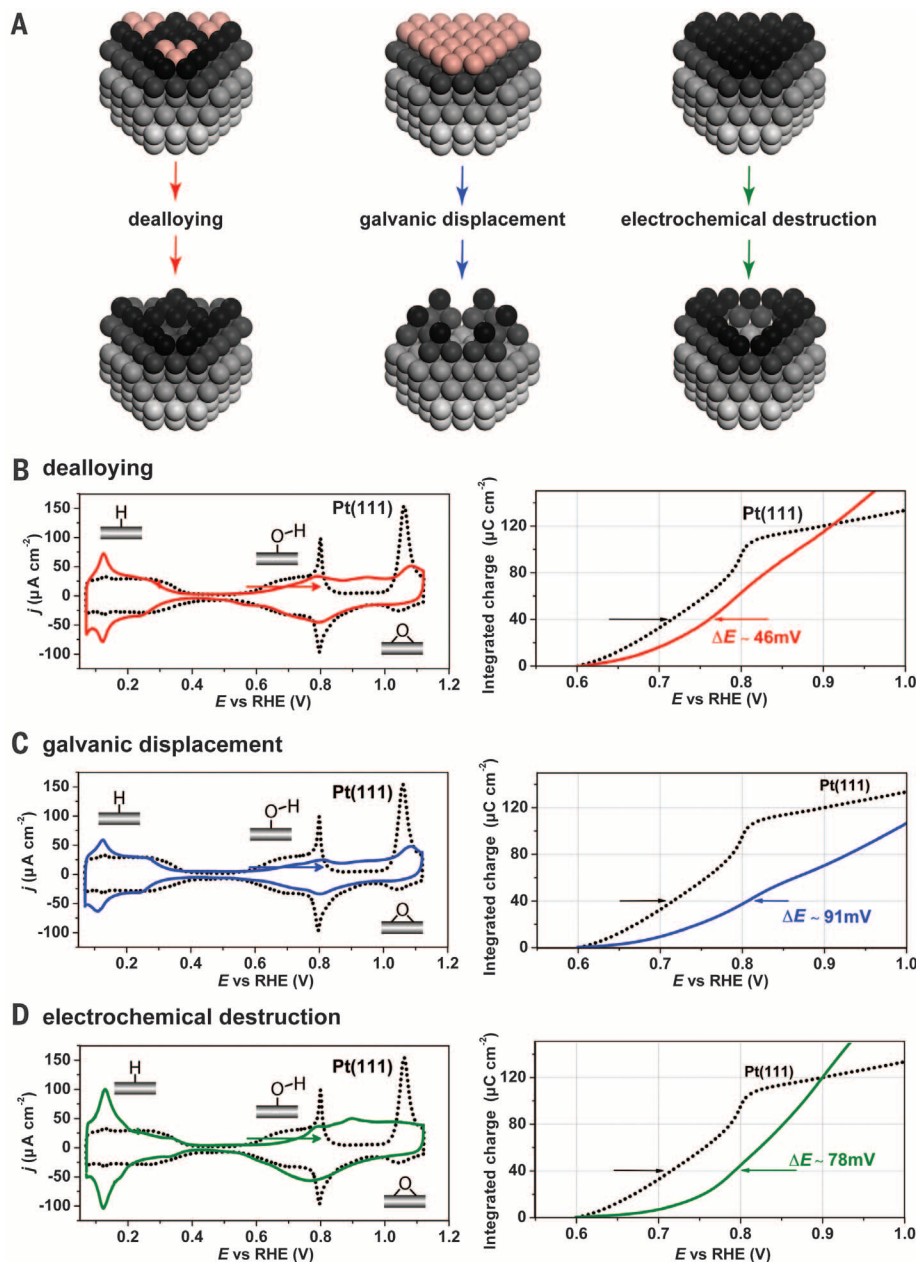


Fig. 3. Electrochemical experiments on pristine and defective Pt(111). (A) Schematics of the approaches used to create defects at Pt(111). Cu atoms appear in red and Pt atoms in gray or black, according to the depth with respect to the surface layer. (B to D) Left: cyclic voltammograms characterizing Pt(111) upon the following electrochemical modifications: (B) dealloying of a Cu/Pt(111) SA (red); (C) five galvanic displacements (blue); and (D) electrochemical destruction of Pt(111) (green). Right: integrated anodic parts of the corresponding voltammograms, scan rate $dE/dt = 50 \text{ mV s}^{-1}$, Ar-saturated 0.1 M HClO₄. The positive shifts indicate weakened *OH adsorption energies and enhanced ORR activity compared to Pt(111).

*OH is transformed into H₂O. Thus, within this model, the ORR activity depends primarily on ΔG_{OOH} and $-\Delta G_{\text{OH}}$. An activity plot is formed when the reaction energies of steps (i) and (ii) are evaluated as a function of a given descriptor. If adsorption energies are used as descriptors, only the optimal adsorption properties will be identified (2, 15, 16). If structural parameters are used as descriptors, the outcome will be the geometry of optimal sites. The choice between energetic and geometric descriptors is determinant, as illustrated in Fig. 2A, where a coordination-activity plot is presented [see also fig. S1 in (26)]. This plot shows that optimal Pt surface sites for the ORR possess $\overline{CN} \approx 8.3$. In agreement with energetic volcano plots (8, 15), ΔG_{OH} on optimal catalysts is ~ 0.15 eV weaker than on Pt(111). Besides, the additional structural prediction ($\overline{CN} \approx 8.3$) can be used to guide experiments.

First, note that $\overline{CN} = 7.50$ for (111) terraces in extended surfaces and sufficiently large NPs (Figs. 1 and 2). If the top of the volcano is found at $\overline{CN} \approx 8.3$, optimal catalytic sites must have more neighbors than (111) terraces. Sites with $cn = 10$ [e.g., bottom of (100) step sites] or $cn = 11$ [e.g., troughs of (110) facets or bottom of (111) step sites] have $\overline{CN}$ values between 8.75 and 9.50, which exceed the optimal value and are problematic because of steric hindrance, weak adsorption energies, and proximity to undercoordinated sites, resulting in adsorbate diffusion to neighboring sites.

If no more first-nearest neighbors can be added to Pt(111) sites, a way of producing sites with $\overline{CN} \approx 8.3$ is by changing the coordination numbers of such first-nearest neighbors—namely, the number of second-nearest neighbors of the active site. Figure 2, B and C, shows two one-layer-deep cavities on Pt(111), corresponding to the removal of six (B) and five (C) adjacent surface atoms. In the configuration in Fig. 2B, the atom in the middle of the cavity (blue) has $cn = 9$, but its six in-plane nearest neighbors (yellow) have $cn = 10$ [one more neighbor compared to Pt(111)] and its three subsurface neighbors (white) have $cn = 12$, so $\overline{CN}_B = (6 \times 10 + 3 \times 12)/12 = 8.00$. Similarly, the active site in Fig. 2C has $\overline{CN}_C = (4 \times 10 + 2 \times 11 + 3 \times 12)/12 = 8.17$. The overpotentials for these sites are lower than on Pt(111) by ~ 0.10 to 0.13 V. Several other configurations may exist, but the design rule is clear, general, and simple: Enhanced Pt(111) sites for the ORR must have an increased number of second-nearest neighbors, so that $cn > 9$ for the first-nearest neighbors of the active site. Such a conclusion could not be obtained using cn as descriptor in Fig. 2, as it does not account for second-nearest neighbors [see fig. S21 in (26)].

We used these theoretical guidelines to engineer active sites at Pt(111) with one of three approaches illustrated in Fig. 3A [see section S2 in (26)]: (i) We stripped away Cu atoms electrochemically from the top layer of an ordered Cu/Pt(111) surface alloy (SA) (9); (ii) we exchanged a deposited Cu-overlayer with Pt ions in solution via galvanic displacement (28) to form Pt “surface islands”; or (iii) we formed a subsurface Pt oxide, which we then reduced during a cathodic poten-

tial scan, causing desorption of some Pt atoms from the surface (29, 30) to create both (desirable) small and (undesirable) large cavities. These approaches create surfaces with different adsorption energies of *OH compared to Pt(111), as shown in Fig. 3, B to D [see also figs. S3 to S5, S8, S10, and S16 to 19 in (26)].

For Pt-based ORR catalysts, the most insightful part of the cyclic voltammograms is the *OH adsorption-desorption region (0.6 to 1.0 V in Fig. 3, B to D). For the three modified electrodes, a noticeable weakening of the *OH adsorption is observed, as the two peaks at ~ 0.8 V appear at more positive potentials. The interaction between *OH and the surface is quantified in the right panels of Fig. 3, B to D. Sizeable positive shifts are observed for dealloyed Cu/Pt(111) SA (~ 46 mV), and for Pt(111) electrodes after galvanic displacement (~ 91 mV) and electrochemical oxidation (~ 78 mV). Thus, these catalysts bind *OH more weakly than pristine Pt(111) (see figs. S6 to S11). Normally, the *OH adsorption potentials predicted from volcano plots compare well to experimental onset potentials for *OH adsorption (15, 17). Here, the experimental shifts in the *OH adsorption peaks (~ 0.05 to 0.09 V) with respect to Pt(111) in Fig. 3 are also in agreement with those in Fig. 2A (0.10 to 0.13 V).

The activities of Pt(111), various (111) defective electrodes, and similarly treated polycrystalline Pt electrodes (Pt_{pc}) presented in Fig. 4 show that specific defects can increase the activity ~ 3.5 times compared to Pt(111). This increase in catalytic activity cannot be explained by the modest increase in the number of accessible surface adsorption sites [maximum 15%; see (26)]. However, defects do not enhance the ORR activity of all Pt electrodes. Figure 4A shows that defects on Pt_{pc} did not noticeably enhance the ORR activity

[see also figs. S13 and S14 in (26)]. This is attributed to different corrosion mechanisms on Pt facets that lead to the formation of dissimilar defects (31).

Because only specific kinds of defects are beneficial, “template” methods generating uniform surfaces with abundant target defects are needed to enhance the ORR activity. This is important for the design of highly active NPs. Convex NPs (Fig. 1) have numerous undercoordinated sites that are not ORR active, and only (111) sites on sufficiently large NPs are similar to extended Pt(111) (see Fig. 2A). Thus, concave geometries are recommendable to introduce sites with $\overline{CN} \approx 8.3$ and enhance the ORR activity (10).

Figure 4B shows the ORR current densities of the most active defective surface in this work and those of state-of-the-art Pt-based catalysts. Pt(111) with cavities possesses ORR activities that exceed those of several active alloys. Therefore, the optimal electronic and coordination configuration of Pt ORR catalysts is close but not identical to that of (111) terraces, and alloying or adding second-nearest neighbors have similar beneficial effects.

Figure S22 in (26) shows the ORR coordination-activity plot for gold, where optimal sites correspond to (100) terraces or possess $\overline{CN} \approx 5.1$. In (26), we outline the extension of $\overline{CN}$ to alloys and other compounds. Coordination-activity plots could be used to model other catalytic reactions such as H₂O₂ production, CO₂/CO reduction, and nitrate reduction, as the relation between adsorption energies and surface coordination exists on metals such as Cu, Ag, and Au and for adsorbates such as *O, *O₂, *H₂O, *H₂O₂, *CO, *CH, *N, and *NO₃[−] (21, 22, 24, 27). Thus, this work opens up the path in heterogeneous catalysis for the design of optimal surface sites utilizing coordination rationales.

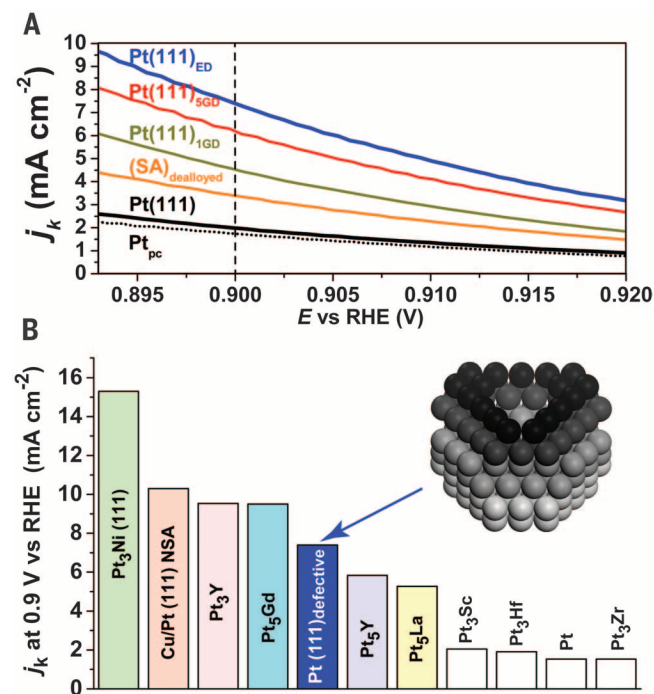


Fig. 4. Comparison between catalysts in this and in previous studies.

(A) Kinetic current densities in O₂-saturated 0.1 M HClO₄ for defective Pt_{pc} (dotted line); Pt(111) (black); dealloyed Cu-Pt(111) SA, (SA)_{dealloyed} (orange); Pt(111) electrodes modified via galvanic displacement, Pt(111)_{IGD} (1 Cu monolayer displaced, green) and Pt(111)_{SGD} (5 monolayers Cu displaced, red); and electrochemical destruction (10 cycles, blue), Pt(111)_{ED}. (B) ORR activities for defective Pt(111)_{ED} and Pt₃Ni (6); Cu-Pt(111) near-surface alloy (17), Pt₃Y and Pt₃Sc (8), Pt₅Gd (12); Pt₅Y, Pt₃Zr, Pt₃Hf, and Pt₅La (15). The potential for comparison is 0.9 V.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6257/185/suppl/DC1
Materials and Methods
Figs. S1 to S22
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SURFACE CHEMISTRY

Identification of active sites in CO oxidation and water-gas shift over supported Pt catalysts

Kunlun Ding,¹ Ahmet Gulec,² Alexis M. Johnson,¹ Neil M. Schweitzer,³ Galen D. Stucky,⁴ Laurence D. Marks,² Peter C. Stair^{1,5*}

Identification and characterization of catalytic active sites are the prerequisites for an atomic-level understanding of the catalytic mechanism and rational design of high-performance heterogeneous catalysts. Indirect evidence in recent reports suggests that platinum (Pt) single atoms are exceptionally active catalytic sites. We demonstrate that infrared spectroscopy can be a fast and convenient characterization method with which to directly distinguish and quantify Pt single atoms from nanoparticles. In addition, we directly observe that only Pt nanoparticles show activity for carbon monoxide (CO) oxidation and water-gas shift at low temperatures, whereas Pt single atoms behave as spectators. The lack of catalytic activity of Pt single atoms can be partly attributed to the strong binding of CO molecules.

Low-temperature catalytic conversions of carbon monoxide (CO) to carbon dioxide (CO₂) via oxidation and water-gas shift (WGS) reactions are integral to several important processes, including the removal of CO from hydrogen gas (H₂) for fuel cell applications (1, 2) and emission control in automobiles with catalytic converters (3). Supported noble metal-based catalysts, mainly platinum (Pt) and gold (Au), have been intensively studied for the reactions during the past decade because of their excellent activity and stability at low reaction temperatures. However, the reaction mechanisms are still highly debated, especially regarding the active site structures—single atoms (4–10) versus small Pt and Au nanoparticles (NPs) (11–16). For instance, there have been disagreements on the promotional effects of alkali cations in CO oxidation (17–20) and WGS (5, 10, 16, 21) reactions over supported Pt and Au catalysts. Some researchers attributed the promotional effects to the increased dispersion and activity of Pt and Au single atoms (5, 10); others correlated it to the increased activity of Pt and Au NPs (16–21). These differences in reaction mechanisms and identification of active sites may have arisen from conclusions drawn from techniques—such as microscopy or x-ray absorption spectroscopy—that provide either statistically limited information or sample-averaged information, respectively. A direct observation of the catalytic performance of specific sites has long been lacking.

Site-specific techniques that provide statistically sufficient information on site identification and quantification as well as the activity evaluation of specific sites would make substantial progress toward resolving these discrepancies. Infrared (IR) spectroscopy of CO on supported noble metal catalysts is widely used because of its sensitivity to the atomic and electronic structures of the binding sites (22–24). Work done by Green *et al.* on titanium dioxide (TiO₂)-supported Au NPs showed that IR spectroscopy could identify the active site in CO oxidation (25) and that CO oxidation occurred within a zone at the perimeter of Au NPs surrounded by a TiO₂ surface. Here, we show that IR spectroscopy with CO as a probe molecule can differentiate and quantify both Pt single atoms and NPs. We confirm the coexistence of Pt single atoms and NPs in many conventional catalysts and show that only the CO molecules adsorbed on Pt NPs can react at low temperatures upon O₂ or H₂O exposure. Thus, the active sites in CO oxidation and WGS reactions are present on the NPs but not on single atoms.

CO molecules were adsorbed on a series of Pt catalysts with varying ratios of Pt single atoms to NPs in order to investigate the corresponding changes to the IR absorption bands. Mesoporous zeolite HZSM-5 [silicon/aluminum (Si/Al) ratio of 62; morphology shown in fig. S1] (26) was chosen as the catalyst support because Al atoms are strictly isolated in the zeolite framework (27), providing isolated binding sites for Pt. The mesoporous structure is introduced so as to facilitate the diffusion of an organometallic Pt precursor, trimethyl(methylcyclopentadienyl)platinum (MeCpPtMe₃), to the Al sites. Pt was loaded on the mesoporous HZSM-5 via either solution grafting at room temperature or vapor deposition at elevated temperatures (26).

The IR spectra of adsorbed CO on four Pt/HZSM-5 samples with different loadings (Fig. 1A) reveal two sets of CO absorption bands centered

¹Department of Chemistry, Northwestern University, Evanston, IL 60208, USA. ²Department of Materials Science and Engineering, Northwestern University, Evanston, IL 60208, USA. ³Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL 60208, USA. ⁴Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106, USA. ⁵Chemical Sciences and Engineering Division, Argonne National Laboratory, Argonne, IL 60439, USA.

*Corresponding author. E-mail: pstair@northwestern.edu

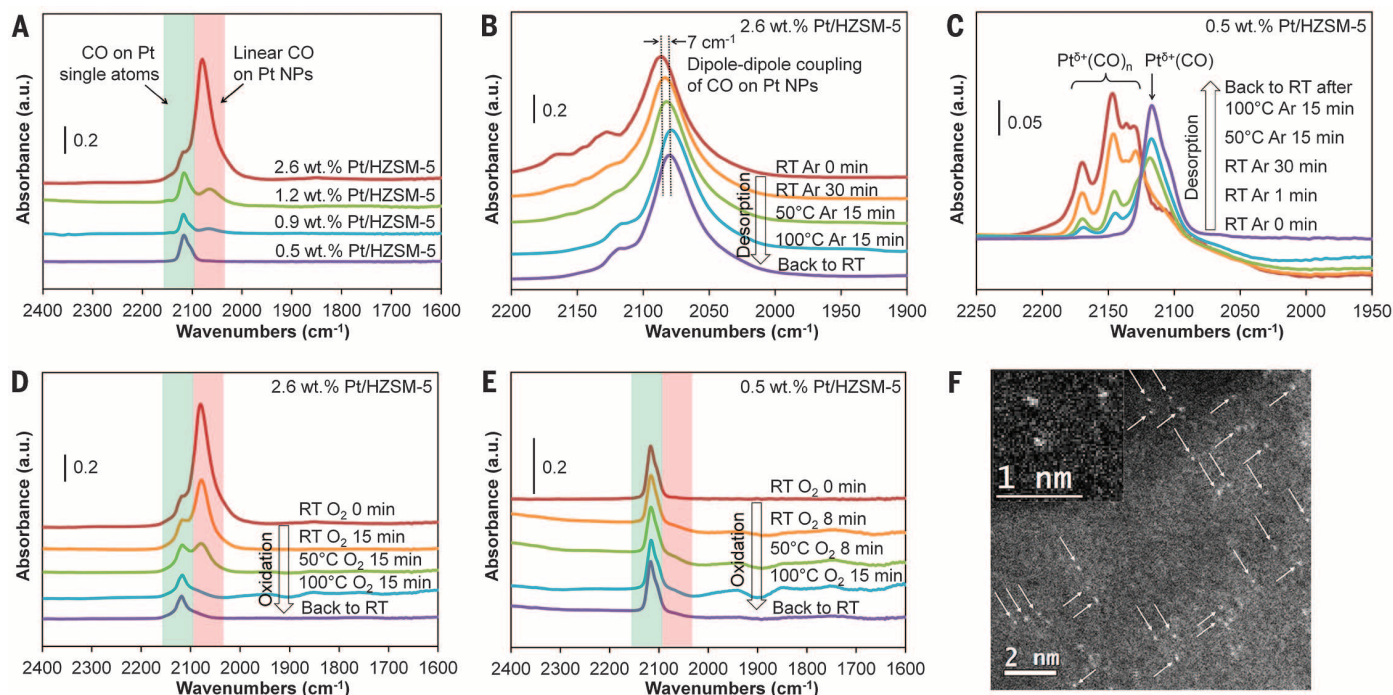


Fig. 1. Spectroscopic and microscopic identification of Pt single atoms and NPs on HZSM-5 and SiO₂ (Al-doped). (A) IR spectra of CO adsorbed on different Pt/HZSM-5 after the desorption processes. (B and C) Time-dependent IR spectra of CO adsorbed on (B) 2.6 wt % Pt/HZSM-5 and (C) 0.5 wt % Pt/HZSM-5 during the desorption process. (D and E) Time-dependent IR spectra of CO adsorbed on (D) 2.6 wt % Pt/HZSM-5 and (E) 0.5 wt % Pt/HZSM-5 during the oxidation process. (F) HAADF image of 0.6 wt % Pt/SiO₂ (Al-doped) with magnified image inserted and arrows to mark the single atoms.

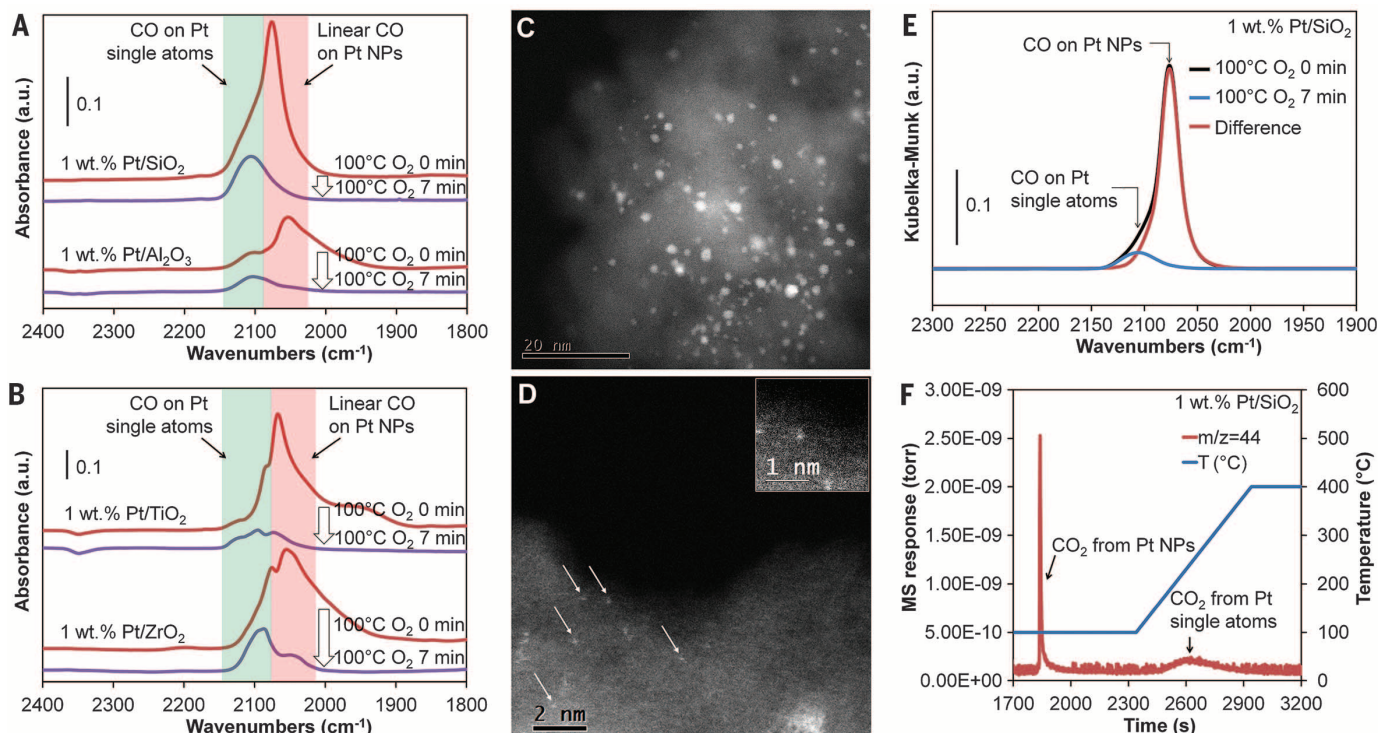


Fig. 2. Identification and quantification of Pt single atoms and NPs on conventional supports. (A and B) IR spectra of CO adsorbed on wet-impregnated (A) Pt/SiO₂ and Pt/Al₂O₃ and (B) Pt/TiO₂ and Pt/ZrO₂ upon O₂ exposure. (C and D) HAADF images of wet-impregnated 1 wt % Pt/SiO₂. Scale bars, 20 nm (C) and 2 nm (D). Arrows indicate the single atoms. (Inset) Magnified image. (E) IR spectra of CO adsorbed on impregnated Pt/SiO₂ before and after O₂ exposure. The difference spectrum shows the CO removed by O₂; Kubelka-Munk unit is used for quantification. (F) CO₂ signal monitored by means of MS during the TPO process of the preadsorbed CO on Pt/SiO₂.

at 2115 cm^{-1} and 2070 to 2090 cm^{-1} . Adsorbed CO was not present on the bare zeolite HZSM-5 sample under our experimental conditions (fig. S2), indicating that these two peaks originate from CO molecules adsorbed on two different Pt species. We assigned the IR peak at 2070 to 2090 cm^{-1} to CO molecules linearly adsorbed in an a-top geometry on Pt^0 atoms on single-crystal or NP surfaces (28–30). The relative intensity of the IR peak at 2070 to 2090 cm^{-1} increased with the Pt loading, which is consistent with the increasing amount of Pt NPs on the zeolite support as observed with transmission electron microscopy (TEM) (figs. S3 and S4). In addition, the redshift of the IR peak at 2070 to 2090 cm^{-1} during desorption (Fig. 1B and fig. S5) correlated with changes in dipole-dipole coupling between CO molecules on a Pt crystal surface (28–30).

A 0.5 weight percent (wt %) Pt/HZSM-5 sample, prepared from room-temperature solution grafting, displayed several CO peaks in the polycarbonyl region (Fig. 1C) (31, 32). These peaks gradually decreased in intensity as the temperature increased during the desorption process (Fig. 1C), and a new peak at 2115 cm^{-1} emerged and eventually became the only peak in the spectrum. The peak transformation was reversed upon reexposure to CO (fig. S6). These trends indicate that cationic $\text{Pt}^{\delta+}$ -polycarbonyl species formed initially upon CO exposure, before transforming into $\text{Pt}^{\delta+}$ -monocarbonyl upon purging and heating. The cationic nature of grafted Pt was confirmed by means of x-ray photoelectron spectroscopy (XPS) (fig. S7).

Compared with the absorption band from CO bonded to Pt^0 NPs, the CO band assigned to $\text{Pt}^{\delta+}$ -monocarbonyl is less redshifted from the gas phase value (2143 cm^{-1}) because of decreased Pt d-electron back-donation to the CO π^* antibonding orbital. At short electron beam exposures, we verified by means of TEM that Pt NPs were not present on the 0.5 wt % Pt/HZSM-5 sample prepared from room-temperature solution grafting, as expected for the absence of the 2070- to 2090- cm^{-1} peak (fig. S3). However, the zeolite structures decomposed in the electron beam within 1 min, leading to the appearance of Pt NPs. The poor stability under electron beam exposure hinders the high-angle annular dark-field (HAADF) imaging of Pt species with atomic resolution in zeolites. In order to avoid the degradation of the support, we used Al-doped amorphous silica as the support. A 0.6 wt % Pt/ $\text{SiO}_2(\text{Al})$ sample prepared from room-temperature solution grafting gave similar CO IR spectra to that of the 0.5 wt % Pt/HZSM-5 sample (fig. S8). Aberration-corrected HAADF images show that the Pt was dispersed on the surface predominantly as isolated single atoms (Fig. 1F and figs. S9 and S10).

On the basis of the IR, TEM, and XPS results, we assigned the IR bands centered at 2115 cm^{-1} and 2070 to 2090 cm^{-1} to CO molecules adsorbed on Pt single atoms and NPs, respectively, which allowed us to compare the oxidation activity of CO molecules adsorbed at different sites and investigate the active species in supported-Pt catalyzed CO oxidation. For all the Pt samples studied,

only the CO molecules adsorbed on Pt NPs could be oxidized and subsequently desorb as CO_2 at reaction temperatures below 100°C. The IR peak at 2115 cm^{-1} , corresponding to CO adsorbed on Pt single atoms, remained unchanged under the reaction conditions (Fig. 1, D and E, and fig. S5). This result clearly indicates the superior activity of Pt NPs as compared with single atoms for CO oxidation.

The coexistence of Pt single atoms and NPs was also observed in a variety of conventional, supported Pt catalysts. The IR spectra of CO adsorbed on 1 wt % Pt/ SiO_2 , Pt/ $\text{Al}_2\text{O}_3(\gamma)$, Pt/ TiO_2 (anatase), and Pt/ ZrO_2 (monoclinic) at 100°C—all of which were prepared by means of incipient wetness impregnation and calcined at 400°C in air—are generally composed of two types of CO bands (Fig. 2, A and B). The major CO bands centered at 2050 to 2080 cm^{-1} are identical to the band from CO adsorbed on Pt NPs shown in Fig. 1A. A redshift of the major IR bands during argon purging was observed as well (fig. S11), implying the nanoparticulate nature of the CO adsorption sites. As well as the major band, each spectrum contains one or more shoulders on the higher frequency side. The band positions of these shoulders are similar, if not identical, to the band position that we have assigned to CO molecules adsorbed on cationic Pt single atoms (within 20 cm^{-1}). These shoulder bands have been observed previously (33–35) but were ambiguously assigned to CO adsorbed on certain cationic Pt species. On the basis of our study with the model Pt/HZSM-5 system,

Fig. 3. ^{12}CO - ^{13}CO exchange on Pt/ SiO_2 . (A) Comparison of the IR peaks of ^{12}CO and ^{13}CO adsorbed on Pt/ SiO_2 before and after O_2 exposure. (B) Shift of the IR peaks at different ^{12}CO exposure time after ^{13}CO adsorption. The four spectra at the bottom were recorded after O_2 exposure in order to show the CO peaks related to Pt single atoms.

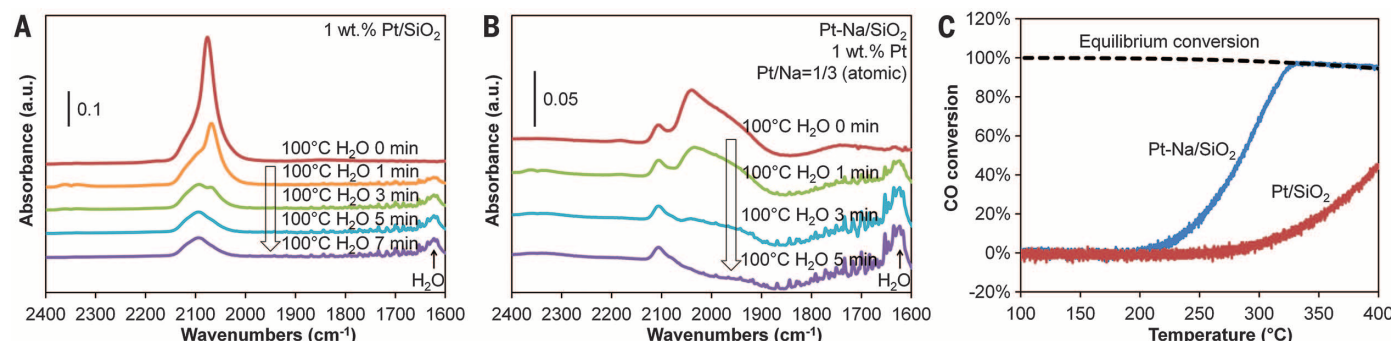
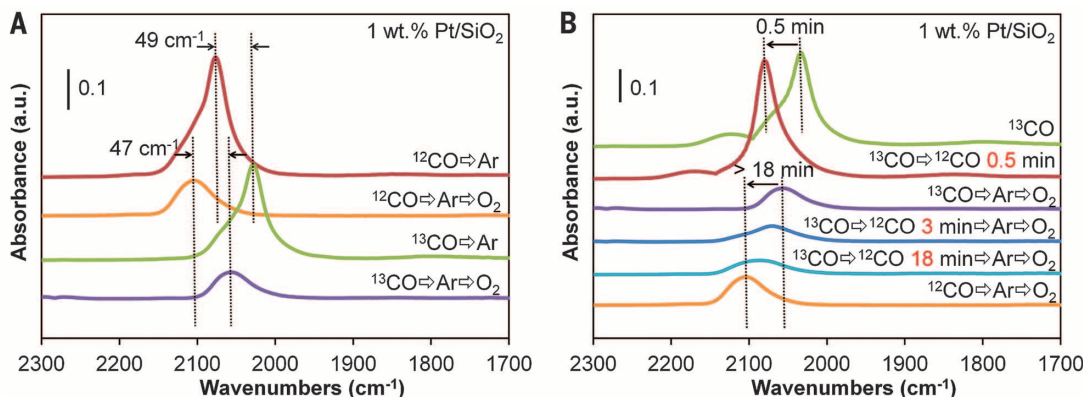


Fig. 4. Catalytic activity of Pt single atoms and NPs in WGS. (A and B) Time-dependent IR spectra of CO adsorbed on (A) Pt/ SiO_2 and (B) Pt-Na/ SiO_2 upon H_2O exposure. (C) Temperature-programmed WGS reaction spectra over Pt/ SiO_2 and Pt-Na/ SiO_2 .

we assigned these bands to cationic Pt single atoms. Similar to the CO oxidation behavior that we observed on Pt/HZSM-5 samples, the CO molecules adsorbed on Pt NPs were quickly oxidized and removed upon O₂ exposure, as indicated by the rapid drop in the major CO bands at 2050 to 2080 cm⁻¹ (Fig. 2, A and B, and fig. S11). Meanwhile, the bands related to CO on Pt single atoms remained unchanged regardless of the identity of the support. Thus, the IR technique can be used for identifying the two Pt species on many conventional metal oxide supports in addition to the model Pt/HZSM-5 system. The coexistence of Pt single atoms and NPs in Pt/SiO₂ was further confirmed by aberration-corrected HAADF imaging, as shown in Fig. 2, C and D, and fig. S12.

Microscopy can provide only statistically limited information about the population of different Pt species. In this regard, IR spectroscopy can be used as a tool for site quantification, with a knowledge of the ratio of IR extinction coefficients for CO molecules adsorbed on Pt single atoms and NPs. To obtain this number, we quantified the respective population of Pt single-atom and NP-related CO adsorption sites in Pt/SiO₂ using temperature-programmed oxidation (TPO) coupled with mass spectrometry (MS) (26). As shown in Fig. 2F (full spectrum and extended discussion given in figs. S13 and S14), the first sharp CO₂ peak is associated with the CO molecules adsorbed on Pt NPs. The second broader CO₂ peak, which extends from 150°C to 350°C, is from the CO molecules that were adsorbed on Pt single atoms, as evidenced from TPO-IR spectra (fig. S15). According to our calculations, the CO molecules adsorbed on Pt single atoms and NPs correspond to ~11 and 10%, respectively, of the total Pt atoms in the 1 wt % Pt/SiO₂ catalyst. From the peak area ratio derived from TPO-MS and IR spectra (Fig. 2, E and F), we obtained a ratio of IR extinction coefficients for CO molecules adsorbed on Pt single atoms and NPs equal to ~0.12. This ratio can then be used for the quantification of CO adsorption sites by using IR spectroscopy.

The temperature-programmed reaction (TPRx) spectra from CO oxidation with O₂ under continuous-flow conditions over Pt/SiO₂ and Pt/Al₂O₃ are shown in fig. S16. The activation energies were measured to be 90 and 95 kJ/mol for Pt/SiO₂ and Pt/Al₂O₃, respectively, which is in good agreement with the experimental and theoretical values reported for supported Pt NPs (30). The TPRx-IR spectra of Pt/SiO₂ (fig. S17) show negligible CO oxidation activity below 150°C because the surface of Pt NPs is dominated by CO adsorption in the presence of CO and O₂. The Pt NP-related CO peak completely disappeared when the reaction temperature was increased to 160°C, accompanied by a constant formation of CO₂ (fig. S17). In contrast, the Pt single atom-related CO peak did not change substantially until the reaction temperature was above 200°C (figs. S15 and S17). The rapid rise of CO oxidation activity is clearly associated with the abrupt change of CO coverage on Pt NPs, which further confirms that the active sites of supported Pt in CO oxidation are Pt NPs but not single atoms.

There are two possible explanations for the distinctly different reactivity of CO molecules adsorbed on the Pt NPs and single atoms. One is the size dependence to O₂ activation by Pt clusters. Small clusters have lower d-band centers, resulting in less electron back-donation to the antibonding orbital of O₂ molecules and thus less efficient O₂ activation (12).

Another possible contribution to the observed reactivity may be the binding strength of the CO molecule to the different Pt species. We conducted a series of ¹²CO-¹³CO exchange experiments on the 1 wt % Pt/SiO₂ catalyst to probe the respective binding strengths of CO molecules on the Pt NP and single-atom sites. The IR bands of ¹³CO adsorbed on Pt single atoms and NPs both redshift ~50 cm⁻¹ compared with those of ¹²CO (Fig. 3A), which is consistent with reported values (28, 29). After the ¹³CO adsorption reached an equilibrium state at 100°C, ¹²CO was introduced to exchange with the preadsorbed ¹³CO. The IR peak related to Pt NPs shifted back to 2080 cm⁻¹ in less than 30 s, indicating that the preadsorbed ¹³CO on Pt NPs was rapidly replaced by ¹²CO. In contrast, the ¹³CO molecules adsorbed on Pt single atoms were only partially replaced by ¹²CO, even after 18 min (Fig. 3B and fig. S18). The stronger binding of CO to Pt single atoms than to Pt NPs resulted in substantially lower catalytic activity for CO oxidation.

We also examined the WGS reaction over the Pt/SiO₂ catalyst in order to compare the reactivity of O₂ and H₂O with adsorbed CO and found that only CO molecules adsorbed on Pt NPs quickly reacted with H₂O, releasing CO₂ (Fig. 4A). The CO molecules adsorbed on Pt single atoms remained intact upon H₂O exposure at 100°C, further emphasizing the lack of activity by the Pt single atoms.

In order to better understand the origin of the promotional effect of alkali cations on Pt single atoms and NPs, we prepared a Pt-sodium (Na)/SiO₂ catalyst with 1 wt % Pt and a Pt/Na molar ratio of 1/3. The WGS performance of Pt/SiO₂ and Pt-Na/SiO₂ that we have observed confirms the promotional effect of Na cations (Fig. 4C). HAADF images show a higher dispersion of Pt in the Pt-Na/SiO₂ case as compared with the Pt/SiO₂ catalyst (fig. S19), which is in agreement with the literature results (5, 10). The IR spectra show that the addition of Na⁺ greatly lowered the CO adsorption peak intensities, implying that Na⁺ could reside in and block CO adsorption sites (36, 37). However, the linear and bridge CO peaks corresponding to Pt NPs of Pt-Na/SiO₂ are redshifted 30 and 90 cm⁻¹, respectively, compared with those of the Na-free sample. At a temperature of up to 300°C, the introduction of H₂O also only removed CO adsorbed on Pt NPs (Fig. 4B and fig. S20). Our IR data show that the promotional effect of Na⁺ in WGS reaction mainly originates from altering the properties of Pt NPs, not single atoms. It also appears that alkali metals could lower the CO surface coverage on Pt NPs. Thus, more sites remain available for the activation of O₂ or H₂O (17, 18).

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S20
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GEOMORPHOLOGY

Erosion by an Alpine glacier

Frédéric Herman,^{1*} Olivier Beyssac,² Mattia Brughelli,¹ Stuart N. Lane,¹
Sébastien Leprince,³ Thierry Adatte,⁴ Jiao Y. Y. Lin,⁵
Jean-Philippe Avouac,³ Simon C. Cox⁶

Assessing the impact of glaciation on Earth's surface requires understanding glacial erosion processes. Developing erosion theories is challenging because of the complex nature of the erosion processes and the difficulty of examining the ice/bedrock interface of contemporary glaciers. We demonstrate that the glacial erosion rate is proportional to the ice-sliding velocity squared, by quantifying spatial variations in ice-sliding velocity and the erosion rate of a fast-flowing Alpine glacier. The nonlinear behavior implies a high erosion sensitivity to small variations in topographic slope and precipitation. A nonlinear rate law suggests that abrasion may dominate over other erosion processes in fast-flowing glaciers. It may also explain the wide range of observed glacial erosion rates and, in part, the impact of glaciation on mountainous landscapes during the past few million years.

Glaciers and icecaps played a major role in shaping the morphology of mid- to high-latitude mountain ranges during the Quaternary period, spanning the past 2.6 million years of Earth's history. Observations suggest that they have also played a fundamental role in the evolution of Earth's climate through a system of positive feedbacks that involves climate, tectonics, and erosion (1–4). Glaciers erode their underlying bedrock mainly through abrasion and quarrying, which theories predict to be proportional to ice-sliding velocity raised to some power (5–7). Numerical models reproduce typical glacial landscape features, such as U-shaped valleys (3, 8), hanging valleys (9, 10), glacial cirques (10, 11), or fjords (12, 13), by implementing these relation-

ships. Despite great advances in the sophistication of these models through the inclusion of high-order ice dynamics (10), subglacial hydrology (10, 11, 13–15), or thermodynamics of water flow (14, 16), they also include poorly constrained parameters. Erosion laws' proportionality constants and velocity exponents are particularly uncertain (5–8, 11, 13).

Estimates of glacial erosion rates ranging from annual to million-year time scales come from monitoring the sediment yield from glacial streams (17–21) and using geochronometric methods (4, 18), respectively. Despite providing key information about the pace at which glaciers may shape mountainous landscapes, these studies have not established an accurate law for glacial erosion. Furthermore, estimates of glacial erosion rates vary by four orders of magnitude from polar to temperate regions on Earth (4, 17, 18). Existing theories do not reproduce such variations. Therefore, our current understanding of the link between climate and glacial erosion suffers from poor constraints on what controls spatial and temporal erosion variability in response to global changes in precipitation and temperature.

We designed this study to specifically constrain how glacial erosion relates to ice-sliding velocity. We simultaneously quantified erosion rates and sliding velocity during a 5-month period, from

November 2013 to April 2014, over the entire Franz Josef Glacier, New Zealand. This glacier exhibits surface velocities that are largely dominated by high sliding velocities on the bedrock (22), up to about 3 m/day. We measured these high velocities accurately from remote sensing and expected to find large erosion rates. The analysis of continuous suspended sediment load indicated very high erosion rates (about 10 mm/year), whereas glacial sediment production remained lower than the transport capacity of the glacial system (23). We also found that the glacial sediments come predominantly from under the glacier, based on the mineralogy, fossil organic carbon, and the very low fraction of modern organic carbon found in the glacial stream (23). These observations imply that sediments collected at the glacier front can be used to constrain the glacial erosion law.

We introduce here a method to measure surface displacement in three dimensions at a 1-m ground resolution and centimetric accuracy, using DigitalGlobe Worldview stereopair images (23). The results confirm fast velocities for most parts of the glacier (Fig. 1) dominated by sliding (22, 23). In addition, we observed similar velocity patterns during the austral summers 2012–2013 and 2013–2014, indicating steady spatial patterns of sliding. Extremely high snow accumulation rates of 4 to 8 m/year (water equivalent) (24) and steep topography account for such high velocities (22).

We exploited the geology of the Southern Alps of New Zealand to determine how erosion varies spatially. This small mountain range resulted from the continental collision between the Australian and Pacific Plates, along a major plate boundary named the Alpine Fault (25), which led to a sharp metamorphic gradient within a 15-km distance (Fig. 2). Rocks adjacent to the Alpine Fault have experienced peak metamorphic temperatures up to about 650°C, whereas rocks about 15 km farther southeast have only experienced 300°C. The Franz Josef Glacier flows almost parallel to this temperature gradient. The rocks are highly fractured but have uniform, steep bedding and foliation (60° to 80°) without kilometer-scale variations in strength or erodability across the catchment. The rocks also contain fossil organic carbon (26), which can quantify the peak metamorphic temperature conditions based on Raman spectroscopy of carbonaceous material (RSCM) (27). By comparing RSCM temperature data in samples collected from

¹Institute of Earth Surface Dynamics, University of Lausanne, CH-1015 Lausanne, Switzerland. ²Institut de Minéralogie, de Physique des Matériaux, et de Cosmochimie, Sorbonne Universités, UMR CNRS 7590, Université Pierre et Marie Curie Paris 06, Muséum National d'Histoire Naturelle, Institut de Recherche pour le Développement UMR 206, 4 place Jussieu, 75005 Paris, France. ³Division of Geological and Planetary Science, California Institute of Technology, Pasadena, CA, USA. ⁴Institute of Earth Sciences, University of Lausanne, CH-1015 Lausanne, Switzerland. ⁵Division of Engineering and Applied Science, California Institute of Technology, Pasadena, CA, USA. ⁶Institute of Geological and Nuclear Sciences, Private Bag 1930, Dunedin, New Zealand.
*Corresponding author. E-mail: frederic.herman@unil.ch

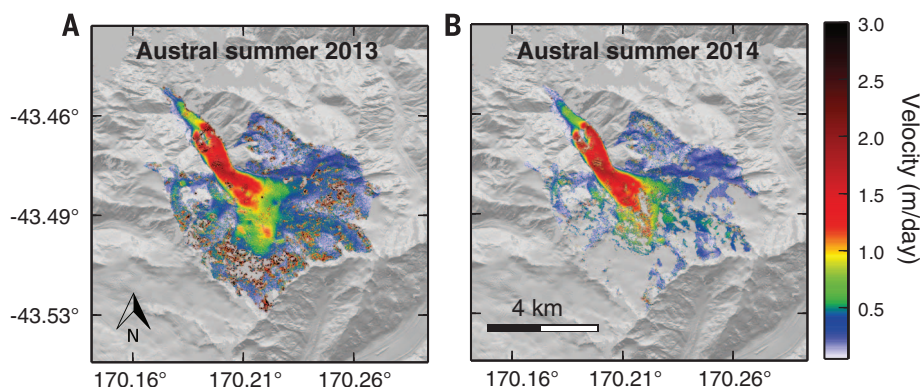
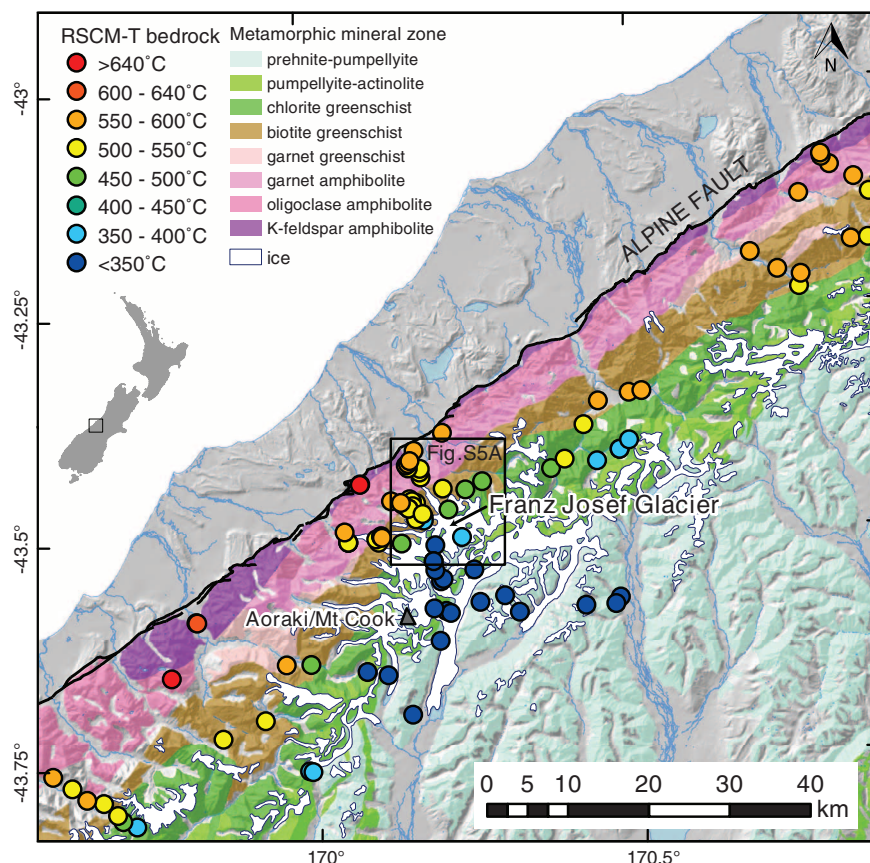


Fig. 1. Franz Josef Glacier surface velocity. The Franz Josef Glacier (Ka Roimata o Hinehukateri in Māori) is located in Westland Tai Poutini National Park on the west coast of New Zealand's South Island. (A) Surface velocity measured in summer 2013 (integrated over 10 days). (B) Surface velocity measured in summer 2014 (integrated over 12 days). The three-dimensional (3D) velocities were derived from the measurements of the 3D displacement derived from Worldview stereo images (23).

Fig. 2. Bedrock metamorphism and RSCM temperature data in the Southern Alps of New Zealand (table S2). A metamorphic map (25) and RSCM temperature data (23) are shown. Both metamorphism and RSCM temperature data show a sharp gradient in the hanging wall of the Alpine Fault. The inset is the location of the Franz Josef Glacier shown in fig. S5, which shows the bedrock temperature model used for the provenance analysis.



the glacial stream, we determined the provenance of the particulate load found in the glacial stream (23). We were then able to reconstruct the magnitude and patterns of erosion.

Our results show erosion patterns closely mimicking the velocity patterns over the 5-month period (fig. S2). Maximum erosion rates occurred around the steeper, faster parts of the glacier. Low erosion rates correspond either to the slowly moving accumulation area or the glacier front. Analysis of the temporal evolution of glacial erosion reveals that instantaneous erosion rates varied by several orders of magnitude over the 5-month period (from about 1 to 500 mm/year; movie S1). Erosion rates were highest in response to large rain events. During that time, water easily reached the ice/bedrock interface to induce an increase of water pressure, glacier sliding velocity, and erosion rates (13, 15, 20, 28, 29). The water discharge data we collected confirm a study showing that the subglacial drainage system does not evolve from a cavity-driven to a channelized system through time (23, 29). These observations explain why we continuously observed such a high sensitivity to water inputs. Furthermore, the integrated erosion rates over the 5-month period compare well to rates integrated over geological time scales (30). This suggests the potential to extrapolate the processes driving erosion during our observations to longer time scales.

We combined the integrated erosion rates with the provenance and remote sensing data to constrain the parameters in an erosion law (Fig. 3A).

We assume that the erosion rate is proportional to the sliding velocity raised to some power (i.e., $\dot{e} = K_g |u_s|^l$, where $\dot{e}$ is the erosion rate, K_g is an erodability constant, u_s is the sliding velocity, and l is an exponent). We constrained K_g and l using two independent methods. The first one is based on the nonlinear least-squares method, and the second is based on Bayesian inversion that enables us to construct the probability density function of the constrained parameters (23). Both approaches lead to a nonlinear relationship, with an exponent l close to 2 (Fig. 3, A, B, and D). This relationship agrees with theoretical predictions for glacial abrasion (5). Abrasion is proportional to the product of the viscous drag force of the ice as it moves on the bedrock and the rate at which debris contained in the ice is dragged against the bed, which both depend on the sliding velocity.

Theoretical models for abrasion and quarrying assume basal sliding to be the primary driver of erosion (5–7). Discriminating which of these two processes dominates is known to be difficult. This is in part because other variables than sliding play a role, including lithological variations or subglacial fluvial activity. One quarrying model (7) accounts for the effect of bedrock strength heterogeneities and variations in water pressure at the ice/bedrock interface. It predicts an erosion exponent $l < 1$ for weak bedrock strength such as that in the Southern Alps. The model also implies decreasing or relatively steady erosion rates with increasing water pressure (7), in contrast to our

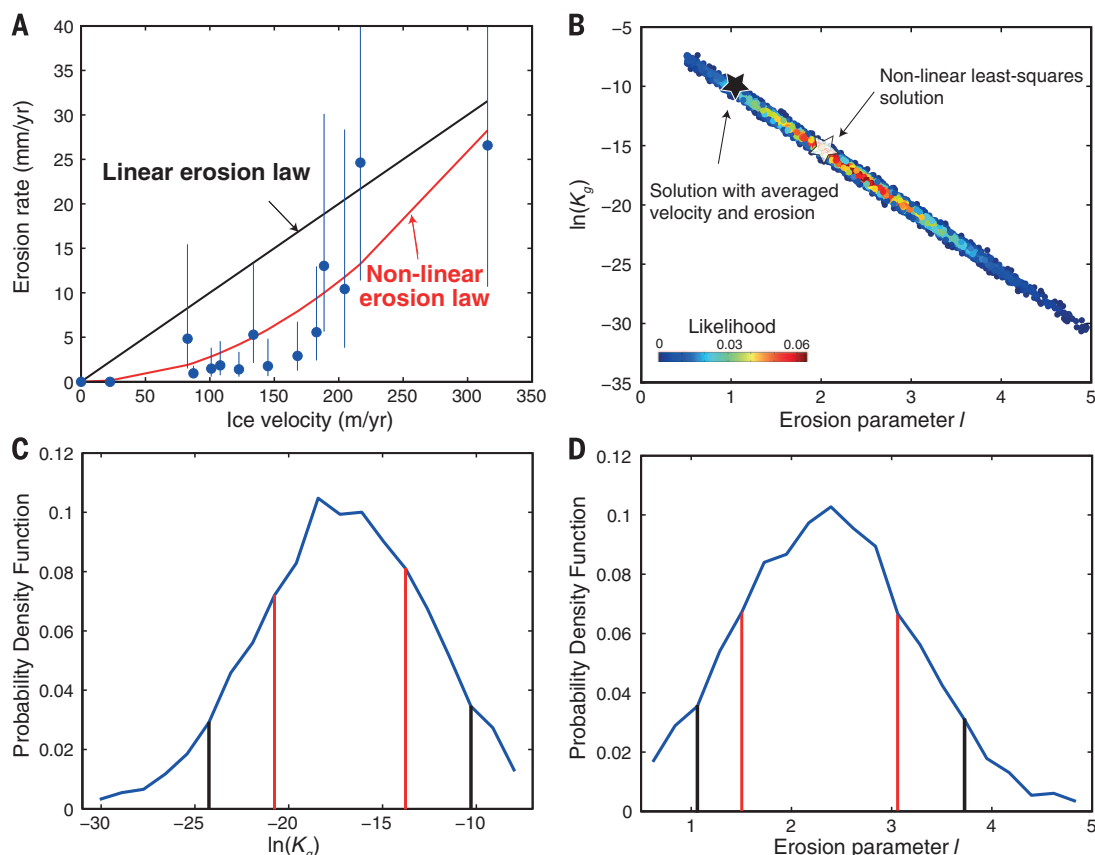
observations of a different relationship (movie S1). This relationship, along with our sliding velocity exponent of about 2, may imply an abrasion-dominated process in the Franz Josef Glacier, although quarrying is required to produce rock fragments that abrade the bedrock.

Existing field estimates in Alaska (19), the European Alps (19), and the Patagonian Andes (18) all suggested an exponent l of 1 and a dimensionless K_g around 10^{-4} . Most landscape evolution models use these values. We observed similar values when spatially integrating erosion rates and sliding velocities for the Franz Josef Glacier. Unfortunately, spatially integrated erosion rates and velocities (19, 20) cannot rigorously constrain K_g and l independently because of the trade-off between the erosion constant K_g and the exponent l (Fig. 3B).

Our observations establish that glacial abrasion is a nonlinear function of ice-sliding velocity. Ice-sliding velocity is to a first order nonlinearly proportional to ice thickness and ice surface slope [eq. 9 in (23, 31)], which are both set by the balance between the glacier mass balance and the divergence of the ice flux [eq. 8 in (23, 31)]. As a result, an increase of ice flux induced by climate change, or increased surface slope, will be accommodated by faster sliding velocities in a nonlinear way. An additional nonlinearity on the sliding exponent in the erosion law (i.e., $l > 1$) thus implies a very high sensitivity of erosion rates to changes in ice flux (23). Therefore, fast-flowing glaciers are likely to be particularly effective at erosion, and

Fig. 3. Constraints on abrasion law.

(A) Erosion rate versus sliding velocity. The blue dots represent measured velocities and integrated erosion rates using a 1-km bin size. Red and black lines correspond to the erosion rate predictions with $l = 2.02$ and $K_g = 2.7 \cdot 10^{-7} \text{ (m}^{-1/2}\text{/year}^{1/2}\text{)}$ and $l = 1$ and $K_g = 10^{-4}$, respectively. The magnitude of the error bars comes from the variability of erosion rates through time. **(B)** Erosion exponent l versus the natural logarithm of erosion constant K_g . Each dot represents sampling of the maximum-likelihood solution, with dots being colored according to their likelihood (from blue to red, with red being most likely) (23). The black star is the estimated value when integrating erosion and velocity over the entire glacier. The white star indicates values obtained with the non-linear least-squares fit (23). The quality of fit to data for each dot in (A) is shown in fig. S2. **(C and D)** Probability density functions for K_g and l , respectively (23). Black and red bars indicate 90 and 60% confidence intervals.



abrasion might become the dominating process over quarrying for fast-flowing glaciers over weak rocks, such as the Franz Josef Glacier.

The nonlinear glacial erosion law may explain why glacial erosion rates span several orders of magnitude, from polar dry regions to temperate alpine glaciers and from soil-mantled hillslope landscapes to steep, tectonically active mountain ranges (4, 17, 18). Atmospheric circulation controls global precipitation, with precipitation increasing from the poles to the equator. In addition, the polar jet stream and its associated westerly winds have a major influence on glacial access to precipitation as they bring moisture onto continents. Several observations suggest that they migrate toward the equator during glacial periods (32). These effects, combined with the nonlinear response of glacial erosion to precipitation changes, would provide an appealing explanation for why the impact of glaciation was more pronounced in mid-latitude regions with steep topography during the Quaternary (4).

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SUPPLEMENTARY MATERIALS

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EDUCATION

Math at home adds up to achievement in school

Talia Berkowitz,* Marjorie W. Schaeffer,* Erin A. Maloney, Lori Peterson, Courtney Gregor, Susan C. Levine,† Sian L. Beilock†

With a randomized field experiment of 587 first-graders, we tested an educational intervention designed to promote interactions between children and parents relating to math. We predicted that increasing math activities at home would increase children's math achievement at school. We tested this prediction by having children engage in math story time with their parents. The intervention, short numerical story problems delivered through an iPad app, significantly increased children's math achievement across the school year compared to a reading (control) group, especially for children whose parents are habitually anxious about math. Brief, high-quality parent-child interactions about math at home help break the intergenerational cycle of low math achievement.

For many families, stories are a regular part of a child's home routine. Parents are motivated to read to their children because they believe this activity promotes children's school achievement. However, they pay much less attention to supporting their children's math learning at home.

A widely held belief among parents is that children's math education is primarily the responsibility of schools and that their role in supporting their children's math learning is not as important as their role in supporting their children's reading (1). This belief is reinforced by messages conveyed through the media and schools, which predominantly focus on the need for parents to interact with their children relating to language and reading (2). Unfortunately, the notion that math education is the purview only of schools and not also of parents ignores the fact that math input in the home is an important predictor of children's mathematical success (3). Here, we demonstrate that a parent-child interactive math app, derived from psychological theories that emphasize the importance of parent involvement in children's learning (4), increases first-grade students' math achievement. Moreover, we show that even a small amount of app usage (once a week) especially helps children whose parents are habitually anxious about math. Given the increasing prominence of tablet-style devices and Internet access (5), this intervention has the potential to be a low-cost, high-benefit method to ensure that parents' uneasiness with math does not translate into their children's low math achievement (6).

Although there is an inherited component to math and spatial thinking (7), experiences, including the math talk that young children hear from their parents, are also implicated in children's mathematics achievement. The amount of num-

ber talk parents engage in with their preschool children predicts 4- and 5-year-olds' grasp of foundational number concepts (3, 8). The frequency with which parents talk about shape and spatial features of objects—using words like circle, tall, edge, and corner—also predicts children's spatial thinking (an important component of mathematical success) as they enter kindergarten (9–11).

If parent math talk is important for children's mathematical success, then adding opportunities for parents and their children to discuss numerical and spatial aspects of math throughout the school year should enhance children's math achievement. It might seem unlikely that a few additional opportunities for math-related talk per week would affect children's math achievement. However, many adults are apprehensive about math, reflected as math anxiety (12), and tend to avoid math whenever possible. Moreover, highly math-anxious parents provide a low quality of math input in the home (6). Therefore, even a modest increase in high-quality parent-

child math talk could boost their children's math achievement.

We recruited a demographically diverse sample of primary caregivers (labeled “parents” for simplicity) and their first-grade children (587 families from 22 Chicago area schools). We focused on early elementary school because students who begin school behind peers in math tend to stay behind in later grades (13). Families were randomly assigned to a math group (420 families) or reading (control) group (167 families), with our main focus, the math group, oversampled. To control for differences in math learning because children attend schools of varying quality, schools with a reading control classroom had at least one classroom assigned to the math group.

Children and their parents were asked to read topical math (or reading) passages and answer corresponding math (or reading) questions, delivered by an iPad app called Bedtime Learning Together (BLT), several times per week over the course of the school year. The math version of BLT is based on Bedtime Math, an app available for free on iTunes and Android. Participating families were given an iPad Mini to access the story problems.

Each passage had five associated questions ranging in difficulty from preschool to late fifth-grade levels. Families did as many questions as desired during each interaction with the app. The reading and math app passages were similar, except the reading passages contained no numerical or spatial content. Math app questions covered topics such as counting fluency, geometry, arithmetic, fractions, and probability; reading app questions dealt with reading comprehension, vocabulary, inference, phonics, and spelling (see the supplementary materials for examples).

By distributing passages with the iPad app, we were able to track how often parents used the app with their children. In addition to app usage, each child's math achievement (14) was assessed at school in a one-on-one session with one of several trained research assistants, both at the beginning (before the iPads were distributed) and at the end of the school year.

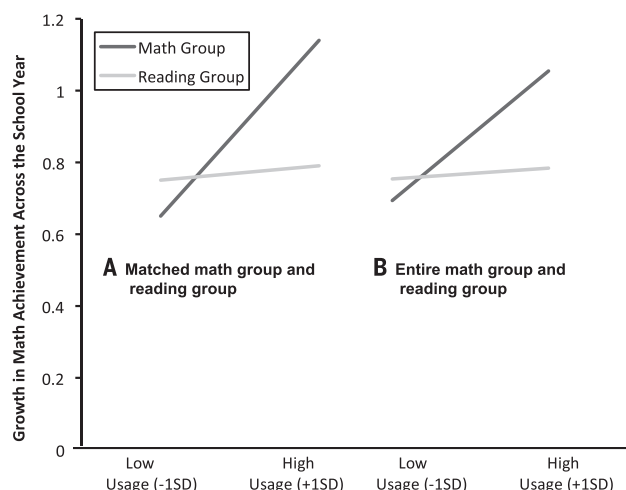


Fig. 1. Estimated number of months of math knowledge children gained across the school year (1 equals 9 months or one school year) as a function of average weekly app use.

University of Chicago, Department of Psychology, Chicago, IL 60637, USA.

*These authors contributed equally to this work. †Corresponding author. E-mail: beilock@uchicago.edu (S.L.B.); s-levine@uchicago.edu (S.C.L.)

Students were randomly assigned to the math or reading group at the classroom level to minimize spillover across treatment conditions. All analyses were conducted using Hierarchical Linear Modeling (HLM) (15) (see the supplementary materials for models) to account for the nesting of students within classrooms.

We began by directly comparing the math achievement of children in the math and reading groups for schools where we had matched math and reading classrooms (math group: $n = 226$ families; reading group: $n = 167$ families). Matching students in this way allowed us to compare math and reading families sampled from the same schools. The more times parents and their children used the app (ranging from 0 to 6.28 times per week), the higher children's math achievement at school year's end (controlling for beginning-of-year math achievement), but only for children in the math group, as shown by a group by use interaction on end-of-year math achievement [$\hat{\beta}_{21} = 4.03, t = 2.83, P = 0.005$] (Fig. 1A and Model S1). This interaction demonstrates that it is not any engagement with parents about academic content that increases children's math achievement but engagement with math specifically.

Looking separately at the math and reading groups, among all families in the math group ($n = 420$ families), the more times children and their parents used the math app, the higher children's end-of-year math achievement (controlling for beginning-of-year math achievement) [$\hat{\beta}_{20} = 2.88, t = 4.01, P < 0.001$] (Model S2). Unlike the math group, a similar model for the reading group showed no significant relation between frequency of app usage and end-of-year math achievement [$\hat{\beta}_{20} = 0.22, t = 0.25, P = 0.81$] (Model S2). More use of the math app (defined as +1 SD above the mean in app use) corresponded to approximately a 3-month math achievement advantage over using the reading app often (Fig. 1, A and B).

If using the math app bolsters children's math achievement because it facilitates parent/child interactions about math, then children whose parents have the most math anxiety and provide lower-quality math input at home (6) should especially benefit from using the math app. Moreover, if parents' math anxiety is linked to variations in how much children grow in math achievement across the school year, then using the math app should decrease or eliminate differences in math achievement growth between children with low-math-anxious parents and children with high-math-anxious parents. Obtaining this latter result would highlight the importance of introducing parent-child math activities at home to ensure that all children (regardless of their parents' level of anxiety and comfort with math) have the opportunity to maximally achieve in math across the school year.

Many adults, in the United States and worldwide, feel at least some apprehension toward math (16, 17). The math app may provide parents with math anxiety an opportunity to talk to their child about math in engaging and effective ways—supporting the kind of math conversations they most likely would not otherwise have. To explore this idea, we assessed parents' math anxiety—their tendency to feel tension, apprehension, or fear in mathematical situations (18). This was done through a math-anxiety questionnaire given to them when they picked up their iPad at the beginning of the school year.

We expected the math achievement of children with high-math-anxious parents to be more affected by use of the math (versus reading) app because these children would not generally be provided with high-quality math input at home (6). Therefore, we first separated parents on the basis of whether they were lower or higher in math anxiety (median split). We then performed an “intent-to-treat” analysis in which we looked at the effect of group (math versus reading app) on children's end-of-year math achievement (controlling for

beginning-of-year math achievement) independent of actual app usage. For children of high-math-anxious parents, we found a significant effect of group, with children in the math group outperforming those in the reading group by almost 3 months in math achievement by school year's end [$\hat{\beta}_{21} = 5.25, t = 1.99, P = 0.048$]. We did not find this same pattern for children of low-math-anxious parents [$\hat{\beta}_{21} = -0.61, t = -0.27, P = 0.79$] (Model S3). An intent-to-treat analysis allows us to rule out factors possibly related to app usage—such as motivation or interest—as explaining our findings.

We next looked at the effect of math app usage, separately, on children whose parents were lower or higher in math anxiety. For children with high-math-anxious parents, we found a significant effect of amount of usage [$\hat{\beta}_{20} = 2.83, t = 3.23, P = 0.002$] (Model S4). For children of low-math-anxious parents, the parallel model also yielded a significant overall effect of app usage [$\hat{\beta}_{20} = 2.76, t = 2.52, P = 0.01$] (Model S4).

To explore these usage effects further, we separated families into three usage groups: Families who had the app but did not use it much (Bin 0: averaging ≤ 0.50 uses per week; $M = 0.22, n = 122$ families); families who used the app on average one time per week (Bin 1: averaging 0.51 to 1.50 uses per week; $M = 1.00, n = 153$ families); and families who used the app on average 2 or more times per week (Bin 2+: averaging 1.51 to 4.30 uses per week; $M = 2.42$ uses per week, $n = 119$ families).

Children of high-math-anxious parents who used the math app about once a week (Bin 1) grew significantly more in math achievement than children of high-math-anxious parents who used the app the least (Bin 0) [$\hat{\beta}_{20} = 8.08, t = 3.14, P = 0.002$] (Fig. 2). However, children of high-math-anxious parents who used the app two or more times a week (Bin 2+) did not show significant growth over children who used the app once a week (Bin 1) [$\hat{\beta}_{20} = 0.52, t = 0.27, P = 0.79$]. If high-math-anxious parents typically provide little and/or low-quality math input in the home, then even a modest amount of high-quality interaction about math should increase the quantity and quality of math input their children receive and therefore boost children's math achievement, as we found.

For children of low-math-anxious parents, the only significant effect was that, at higher doses of math-app use (Bin 2+), these children grew significantly more in math achievement than those who interacted with their parents relating to the app less often (Bin 1) [$\hat{\beta}_{20} = 7.31, t = 2.81, p = 0.006$]. There was a slight dip in performance between those who used the app the least (Bin 0) and those who used it once a week (Bin 1), although this difference was not significant [$\hat{\beta}_{20} = -4.35, t = -1.61, P = 0.11$]. Additionally, there was no significant difference between those who used the app the least (Bin 0) and those who used it the most (Bin 2+) [$\hat{\beta}_{20} = 1.60, t = 1.02, P = 0.31$]. There is likely less of a meaningful usage effect for children of low-math-anxious parents because these parents are already providing rich math input at home (see the supplementary materials for comparable reading group analyses).

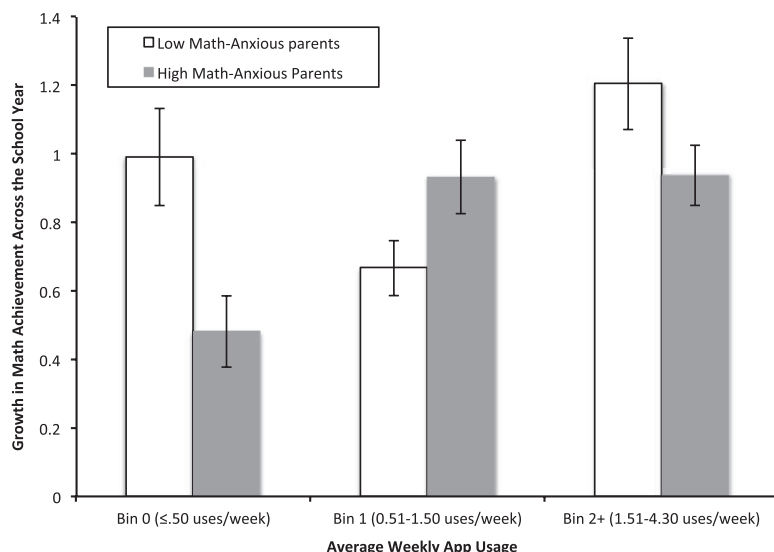


Fig. 2. Number of months of math knowledge children gained across the school year (1 equals 9 months or one school year) as a function of average weekly app use and parents' math anxiety.

When families in the math group used the app the least (Bin 0), children with high-math-anxious parents grew significantly less in math achievement by the end of the school year relative to children with low-math-anxious parents [$\beta_{20} = -7.94$, $t = -2.42$, $P = 0.02$] (Fig. 2 and Model S5). Strikingly, using the math app mitigated this negative relation between parents' math anxiety and children's math achievement. When families used the app on average once a week or more, children with high-math-anxious parents made gains in math achievement by the end of the school year that did not significantly differ from those made by children with low-math-anxious parents {Bin 1: [$\beta_{20} = 3.44$, $t = 1.53$, $P = 0.13$]; Bin 2+: [$\beta_{20} = -3.60$, $t = -1.21$, $P = 0.23$]} (Model S5).

Thus, when parents and children interact about math story problems—even as little as once a week—children show increased math achievement by the end of the school year. The benefits of occasional math-related interactions are especially apparent for children whose parents are anxious about math. By providing an engaging way for math-anxious parents to share math with their children, the math app may help cut the link between parents' high math anxiety and children's low math achievement (6).

The current findings are of particular relevance in view of the multibillion-dollar educational app market (19). Scant research exists on the effectiveness of apps marketed as educational (20), and the research that has been done does not always find benefits for children's learning. Use of enhanced e-books that target literacy can actually be detrimental to children's basic reading comprehension when they contain distracting sounds and animations (21). The math app used here has several specific features that may have contributed to its effectiveness. First, it was basic in nature (very few sounds, animations, or videos) to avoid distracting elements. Second, it was designed to align with the goals of the Common Core Standards at varying grade levels. Third, it was designed to be used by parents and children together, based on the known importance of early parental input, and specifically parent math talk, for children's achievement. The app may give parents—especially high-math-anxious parents or even parents with less skill or interest in engaging in math—more and better ways to talk to their children about math not only during app usage but also in other everyday interactions. We have shown that using this math app enhances the likelihood that children will succeed in math, which is essential for academic success and for the robustness of the science, technology, engineering, and math pipeline.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6257/196/suppl/DC1
Materials and Methods
Supplementary Text
Fig. S1
Table S1
Models S1 to S8
Accessory Data File S1

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PLANT SCIENCE

Visualization of cellulose synthases in *Arabidopsis* secondary cell walls

Y. Watanabe,^{1,2} M. J. Meents,^{1,2} L. M. McDonnell,^{2*} S. Barkwill,² A. Sampathkumar,³ H. N. Cartwright,⁴ T. Demura,⁵ D. W. Ehrhardt,^{4,6} A. L. Samuels,^{1†} S. D. Mansfield^{2‡}

Cellulose biosynthesis in plant secondary cell walls forms the basis of vascular development in land plants, with xylem tissues constituting the vast majority of terrestrial biomass. We used plant lines that contained an inducible master transcription factor controlling xylem cell fate to quantitatively image fluorescently tagged cellulose synthase enzymes during cellulose deposition in living protoxylem cells. The formation of secondary cell wall thickenings was associated with a redistribution and enrichment of CESA7-containing cellulose synthase complexes (CSCs) into narrow membrane domains. The velocities of secondary cell wall-specific CSCs were faster than those of primary cell wall CSCs during abundant cellulose production. Dynamic intracellular trafficking of endomembranes in combination with increased velocity and high density of CSCs, enables cellulose to be synthesized rapidly in secondary cell walls.

Cellulose, the most abundant biopolymer on Earth, is a key biomechanical component of land plants and a valuable natural resource. Cellulose in the primary cell wall, which is laid down during plant growth,

determines plant shape (1). However, the bulk of terrestrial biomass is composed of the cellulose in secondary cell walls, which are laid down after the cell has stopped growing to strengthen plant vasculature and structure (2). The strength of these walls is derived from the organization of cellulose microfibrils, which, relative to primary cell walls, possess cellulose with a higher degree of polymerization, increased microfibril crystallinity, and a higher degree of microfibril organization (2, 3).

Cellulose is synthesized at the plasma membrane by cellulose synthase (CESA) enzymes that are organized in multiprotein cellulose synthase complexes (CSCs) (4). In *Arabidopsis thaliana*, 10 CESA isoforms exist, with CESA1, CESA3, and CESA6 involved in primary cell wall synthesis

¹Department of Botany, University of British Columbia, Vancouver, BC, Canada. ²Department of Wood Science, University of British Columbia, Vancouver, BC, Canada. ³Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA. ⁴Department of Plant Biology, Carnegie Institution for Science, Stanford, CA, USA. ⁵Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan. ⁶Department of Biological Sciences, Stanford University, Stanford, CA, USA.

*Present address: Division of Biological Sciences, University of California, San Diego, CA, USA. †Corresponding author. E-mail: annelacey.samuels@botany.ubc.ca (L.S.); shawn.mansfield@ubc.ca (S.D.M.)

(5, 6), and CESA4, CESA7, and CESA8 required for secondary cell wall production (7). At least three distinct isoforms are required for normal cellulose production (5–7). Live-cell imaging of primary cell wall CSCs clearly shows CESA velocity and distribution at the plasma membrane as well as intracellular trafficking (8–10). Secondary cell walls are produced in vasculature and fibers deep within plant tissues, limiting the resolution of live-cell imaging (11, 12). Here, we visualized cellulose synthesis in secondary cell walls using fluorescently tagged CESA7 in a unique system where live epidermal cells are induced to form secondary cell walls ectopically (13).

Arabidopsis plants were engineered to constitutively express a transcription factor controlling xylem tracheary element cell fate, VASCULAR NAC-DOMAIN7, fused to a glucocorticoid receptor (VND7::GR). When these plants are exposed to a glucocorticoid hormone such as dexametha-

sone, cells are induced to become protoxylem-like tracheary elements (13). After induction, the secondary cell wall cellulose synthase genes were transcriptionally up-regulated by a factor of 1.5 to 3.0, whereas primary cell wall-related cellulose synthase genes were transcriptionally unaffected (table S1). Although it is not possible to know whether the VND7::GR levels in these plants are comparable to endogenous VND7 in protoxylem tracheary elements, the induced cells have features typical of developing protoxylem, such as secondary cell wall thickenings alternating with primary cell wall domains, in spiral or annular patterns. To investigate cellulose deposition, we crossed plants carrying the VND7::GR induction system with *cesa7/irx3-4* null mutants complemented with a functional fluorescently tagged CESA7 (YFP::CESA7) driven by its native promoter (fig. S1). Fluorescent signal from CSCs was detectable in induced epidermal cells, although identification

of individual plasma membrane-localized CSCs was possible only with the use of an optimized spinning disk confocal imaging system (optimization parameters are described in fig. S2).

Use of this optimized system permitted the visualization of discrete YFP::CESA7 particles in the plasma membrane of induced protoxylem tracheary elements (Fig. 1). Their linear movement at slow and steady velocities meets the criterion of actively synthesizing CSCs (movie S1) (8–10, 14). CSCs moved in a bidirectional fashion along plasma membrane domains underlying secondary cell wall thickenings. Relative to labeled primary cell wall CSCs (8–10), secondary cell wall CSCs showed a higher density at the plasma membrane, with overlapping signals from individual particles largely indistinguishable over extended areas (Fig. 1, B and C, and fig. S3). Moreover, CSC distribution changed over the course of secondary cell wall development. Early in development,

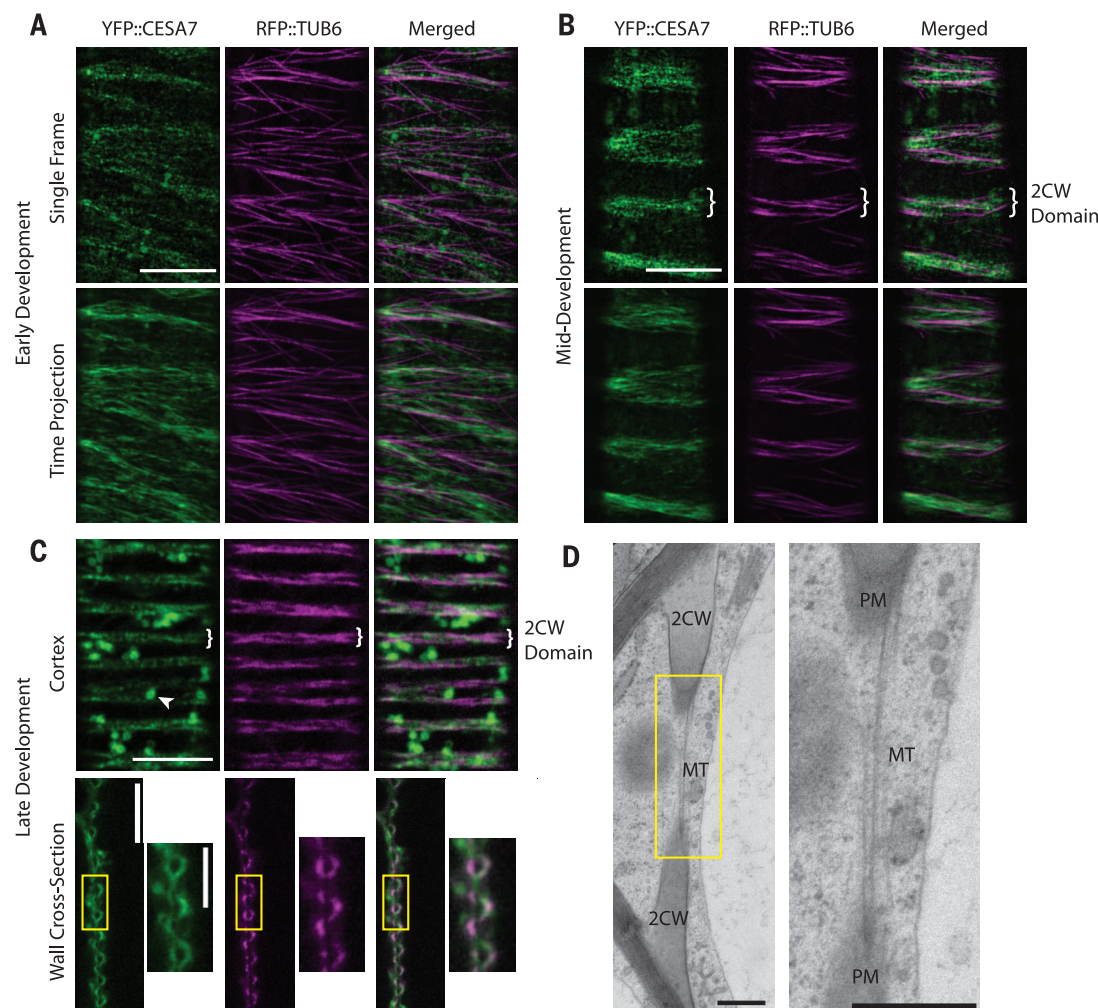


Fig. 1. CESA7 and microtubules are constrained to secondary cell wall domains during protoxylem development. (A) Early development: YFP::CESA7-labeled CSCs and RFP::TUB6-labeled microtubules localize diffusely across the plasma membrane. (B) Mid-development: Narrow microtubule bundles lie underneath tight tracks of membrane-localized CSCs. (C) Late development: Apparent concentrations of YFP::CESA7 and RFP::TUB6 at

the edges of forming secondary cell walls are revealed as uniform signal in subcortical optical sections. YFP::CESA7-labeled Golgi (arrowhead) are visible because of the increased depth of imaging through secondary cell wall thickenings. (D) TEM micrographs highlight plasma membrane (PM) curvature over secondary cell walls (2CW), lined by cortical microtubule (MT) bundles. Scale bars, 10 μ m [(A) to (C)], 500 nm (D).

CESA7 signal was observed across the plane of the plasma membrane on tracks defined by bands of microtubules forming in the cell cortex (Fig. 1A, merged image, and table S2). As secondary cell wall synthesis progressed (Fig. 1B), CESA7 signal was enriched in regions of the plasma membrane associated with tight bundles of microtubules (Fig. 1B and table S2) (15). In late secondary cell wall development (Fig. 1C), the YFP::CESA7 signal was apparent as U-shaped plasma membrane furrows curving around the secondary cell wall thickenings. The majority of the CESA7 signal was evenly distributed and restricted to these curved domains (Fig. 1C, inset). When VND7::GR-induced cells were cryofixed and examined by transmission electron microscopy (TEM), tangential sections along the cell surface revealed the curved domain of plasma membrane around secondary cell walls, as well as their associated microtubules in the cell cortex (Fig. 1D).

The rate of cell wall deposition is the consequence of both the concentration of CSCs and the rate at which each CSC produces cellulose. The importance of the restriction of the CSCs to the curved membrane domains is that cellulose deposition is concentrated in a discrete area of

intense production. Such concentration may help to explain why secondary cell walls are synthesized more quickly than primary cell walls (16). With live-cell imaging, we measured deposition rates of individual CSCs. We assumed that CSC movement through the plasma membrane is a function of glucan chain biosynthesis and its subsequent crystallization into cellulose microfibrils, such that faster CSC velocities would reflect faster rates of synthesis (3, 17). To facilitate this comparison, we quantified the velocities of YFP::CESA7-labeled CSCs at the cell membrane and primary cell wall CSCs (GFP::CESA3) (9, 10) under identical growth and imaging conditions, alternating data collection between plant lines in an imaging session to control for possible environmental effects (Fig. 2). Time projections over a 5-min data collection period showed CSC tracks across the plasma membrane (Fig. 2, A and B). These tracks defined lines for kymograph analysis of particle velocity. In primary cell wall CSC kymographs, the tracks of each GFP::CESA3 were distinct (Fig. 2A). The tracks of CESA7 were more difficult to discern because of their higher density (Fig. 2B and fig. S3). The slope of the lines in the kymograph (Fig. 2, A and B, arrows) represents

the CSC velocities, with steeper slopes indicating faster CSC movement (more displacement on the spatial axis). The average velocity of CESA7-containing CSCs producing secondary cell walls was 265 ± 75 nm/min [mean $\pm$ SD, $n = 36$ cells (40 CSCs per cell) from 12 plants] (Fig. 2D). Velocity changed over the course of protoxylem differentiation, which was divided into stages according to microtubule banding and secondary cell wall features. Average CESA7 velocity was 293 ± 29 nm/min during early development, 327 ± 37 nm/min during mid-development, and 187 ± 38 nm/min during late development (Fig. 2E). In contrast, primary cell wall CESA3-containing CSCs displayed an average velocity of 231 ± 34 nm/min ($n = 12$; 4 cells from 4 plants; Fig. 2C). Analysis of variance (ANOVA) identified significant variation among conditions ($F_{3,44} = 38.7$, $P = 2.133 \times 10^{-12}$). Post hoc Tukey's pairwise comparison tests indicated that primary cell wall CSC velocity was significantly different from all stages of secondary cell wall development. Secondary cell wall CSC velocity did not differ significantly between early and mid-development, but both were significantly different from late development ($P < 0.01$; Fig. 2E). This illustrates that

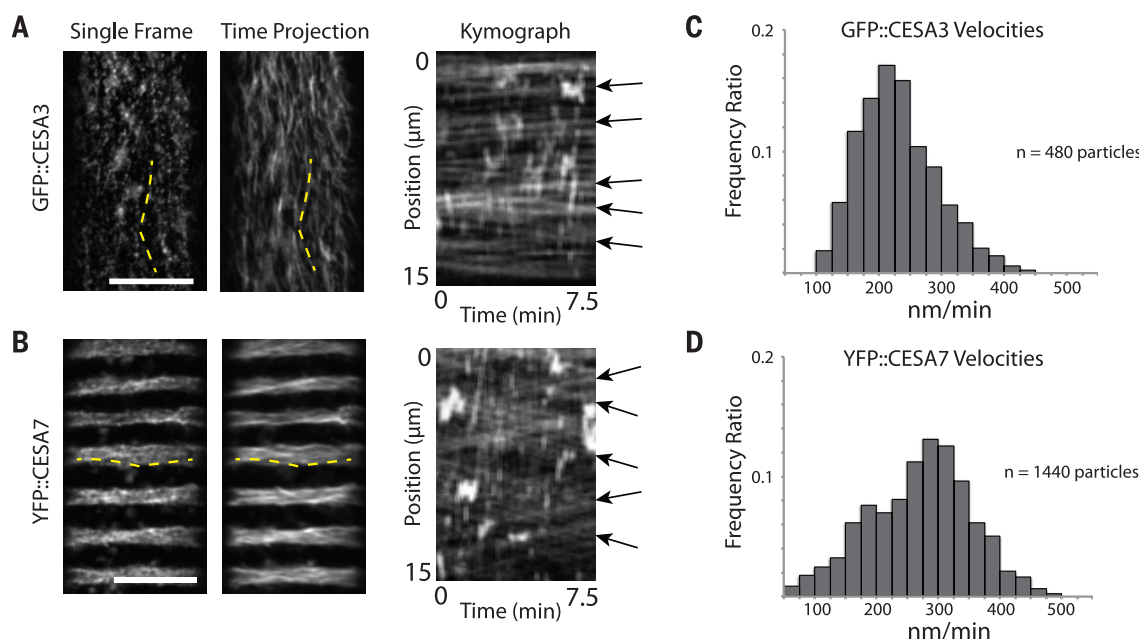
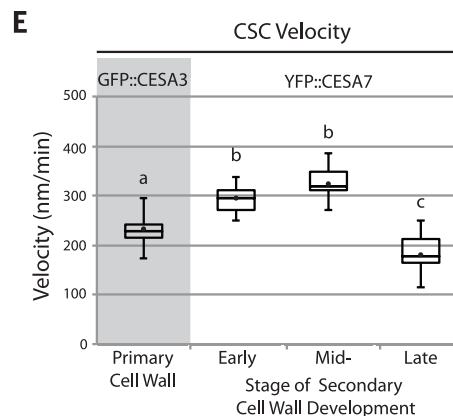


Fig. 2. Secondary cell wall CSCs have a higher velocity than primary cell wall CSCs during peak cellulose deposition. (A and B) CSC tracks in single frames and time projections for primary cell wall (A) and secondary cell wall (B) visualized using GFP::CESA3 and YFP::CESA7, respectively. Kymographs sampled along the yellow lines show CSC trajectories over time, from which CSC velocity was calculated. Scale bars, 10 μ m. (C and D) Histograms of GFP::CESA3 (C) and YFP::CESA7 (D) velocities calculated from kymograph analysis. (E) Box plot of CSC velocities across stages of xylem cell development. Means with different letters represent statistically significant differences (Tukey's pairwise comparison, $P < 0.01$). For each developmental stage, 480 CSC velocities were measured from 12 cells in four plants. In (D), 1440 velocities were pooled from all developmental stages. In (E), velocities were averaged for each cell before analysis.



secondary cell wall CSCs move more rapidly in the plane of the membrane during peak secondary cell wall synthesis, then slow as the cell nears maturity, prior to programmed cell death. Thus, both the high density and high velocity of synthesis by individual CSCs contribute to rapid cell wall synthesis during secondary cell wall formation.

Previous studies examining fluorescently tagged secondary cell wall CESAs, visualized through layers of tissue to the center of the root, could not resolve CSCs at the plasma membrane, although these authors made contributions to understanding the trafficking of CSCs in intracellular endomembranes (11, 12). The authors described “CESA7-containing organelles” actively streaming through

the cytoplasm and pausing at domains of secondary cell wall deposition (12). However, because of technical limitations, the nature of the organelles was not clear. In the induced protoxylem tracheary element system of the VND7::GR lines, we found that the CESA7-containing organelles were both small CESA-containing compartments (SmaCCs) (10) and Golgi (Fig. 3; red arrows in movie S2). SmaCCs were both Golgi-independent SmaCCs (blue arrows in movie S3) and Golgi-associated SmaCCs (yellow arrows), and their behaviors were most easily identified using fluorescence recovery after photobleaching (FRAP) (Fig. 3, A and B, and movies S3 and S4). After bleaching the bright plasma membrane-localized CSC signal, both SmaCCs and Golgi repopulated

the underlying cytoplasm (Fig. 3A). Golgi and SmaCCs often moved in a coordinated fashion (movie S3; $40 \pm 15\%$ of SmaCCs were associated with Golgi, $n = 3$ cells), and frequently Golgi and associated SmaCCs would pause (ranging from 15 s to 3 min) at secondary cell wall domains, consistent with previous observations (12). When the Golgi moved on, a CSC signal would persist and split within 70 ± 20 s ($n = 8$ events; Fig. 3B and movie S4). This stationary signal followed by slow steady movement (280 ± 30 nm/min, $n = 16$ particles) fits the criterion for an insertion event of multiple CSCs into the plasma membrane, as defined for primary cell wall CSCs (10). We were unable to quantify the number of insertion events at secondary cell wall domains. However, it was

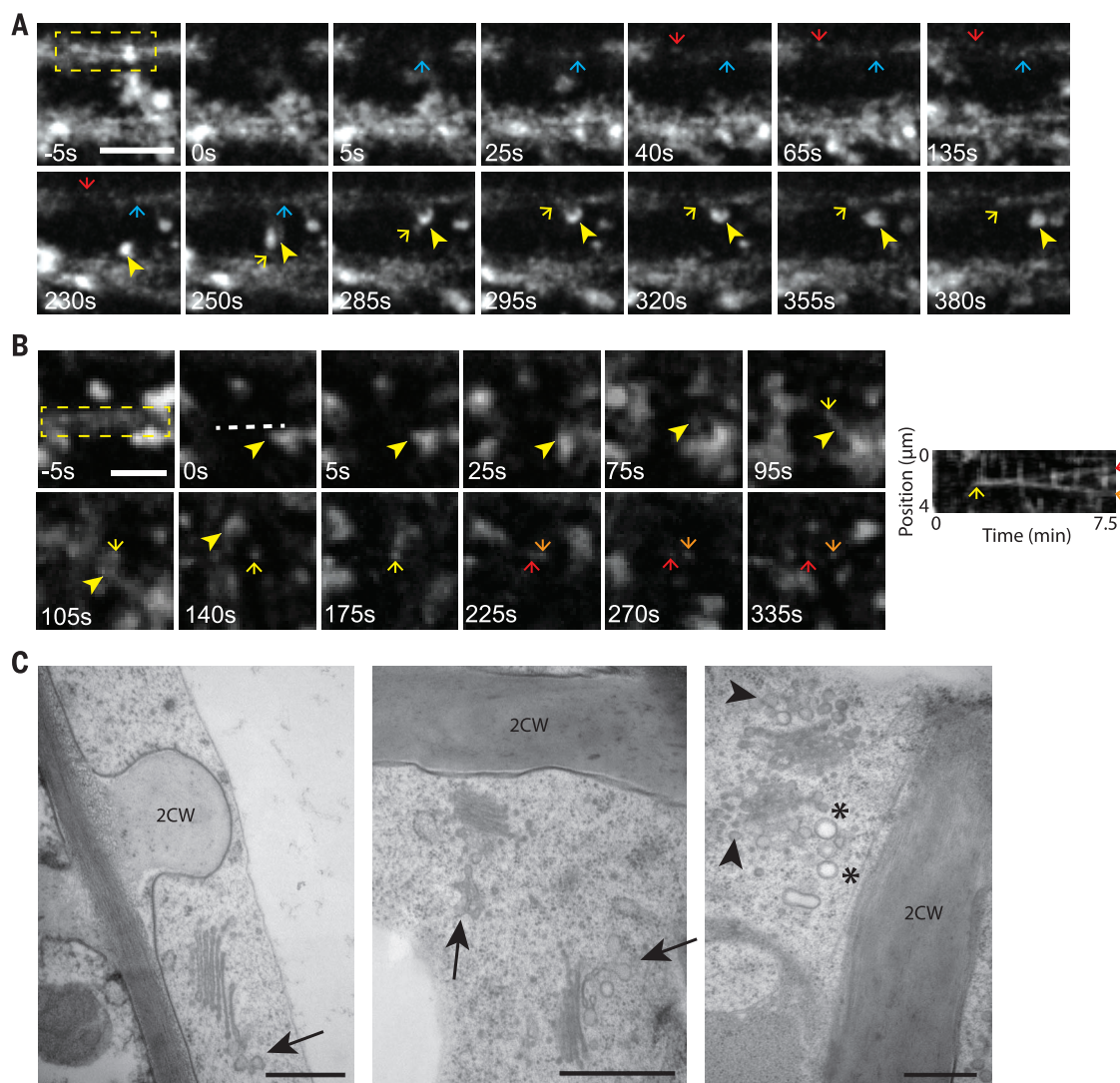


Fig. 3. Golgi and SmaCCs densely populate and rapidly deliver CSCs to domains of secondary cell wall formation. (A) Fluorescence recovery after photobleaching (FRAP) of YFP::CESA7 in the boxed area, overlying a secondary cell wall thickening. Abundant Golgi-independent SmaCCs (red and blue arrows) rapidly repopulate bleached regions. Additionally, Golgi (arrowhead) and closely associated SmaCCs (yellow arrow) can be seen moving from one secondary cell wall band and pausing at another. (B) FRAP and kymograph analyses demonstrating insertion at the plasma

membrane of at least two YFP::CESA7-labeled CSCs from a SmaCC (yellow arrow). After the Golgi (arrowhead) moves away, the signal from the SmaCC splits into two distinct punctae with steady velocities (red and orange arrows). (C) TEM micrographs of cytoplasm around secondary cell walls showing a diversity of closely associated vesicles. Trans-Golgi networks (arrows), secretory vesicle clusters (arrowheads), and electron-lucent vesicles (asterisks) are indicated. Scale bars, 5 μ m (A), 2.5 μ m (B), 500 nm (C).

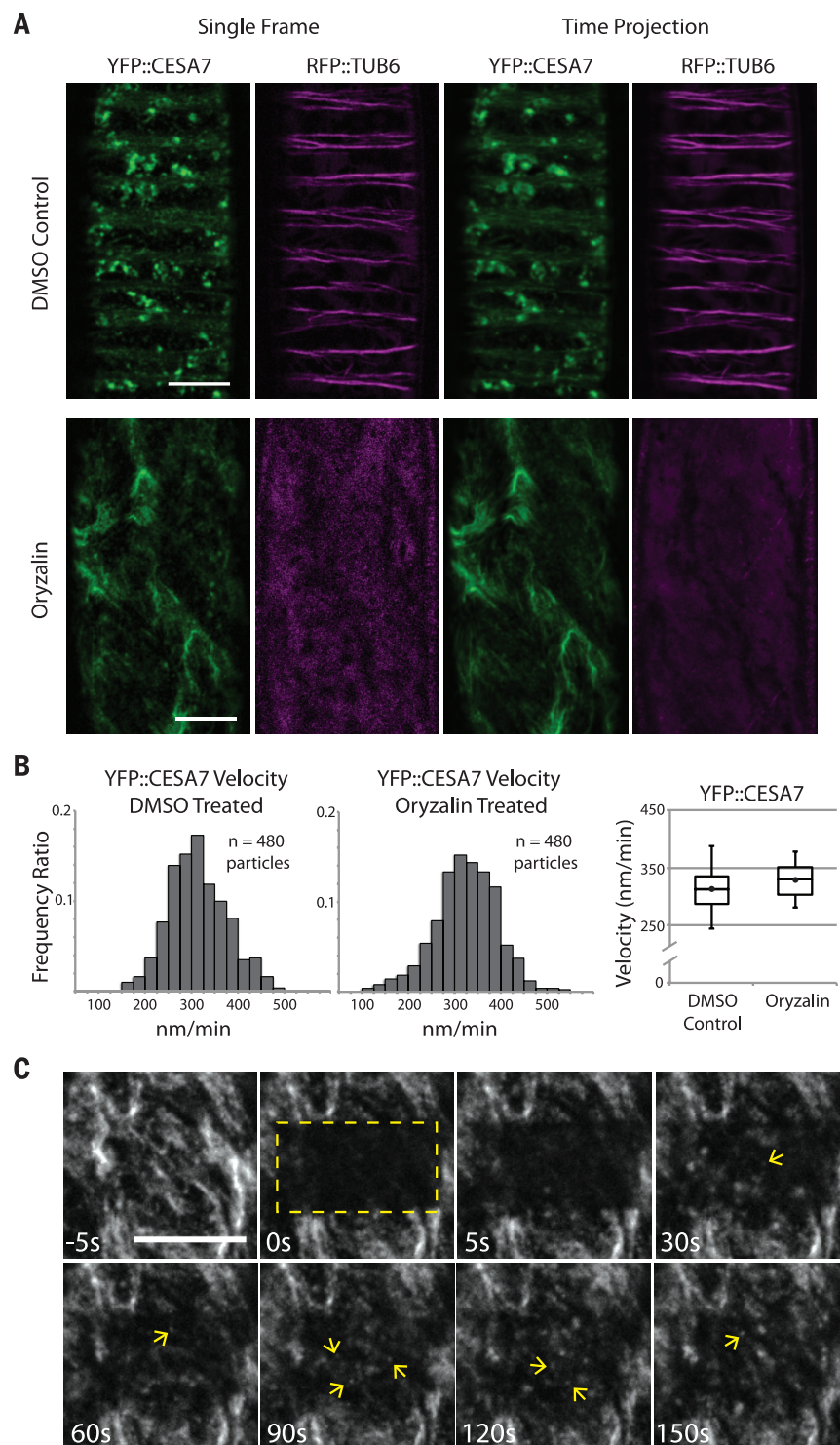


Fig. 4. Secondary cell wall CSC distribution at the plasma membrane is disorganized by the loss of microtubules after oryzalin treatment while CSC delivery and motility are unaffected. (A) VND7::GR-induced cells expressing YFP::CESA7 and RFP::TUB6 after oryzalin treatment. Plasma membrane-localized secondary cell wall CSCs follow aberrant tracks in the absence of microtubules. (B) CSC velocities were not significantly different between oryzalin- and DMSO-treated cells (Student's *t* test, $P = 0.49$). For each condition, 40 CSC velocities were averaged for each of 12 cells in four plants. (C) FRAP of YFP::CESA7 signal revealed insertion of CSCs at the plasma membrane after microtubule loss (arrows). Scale bars, 10 μm [(A) and (B)].

apparent that CSC insertion events were restricted to thickenings (in 12 photobleached regions of 10 μm^2 in six cells, no CSC inserted in plasma membrane regions between thickenings), hence the targeting of these events is tightly limited to secondary cell wall domains. TEM of induced developing protoxylem cells revealed several vesicle populations associated with Golgi that may carry CSCs, including trans-Golgi networks, secretory vesicle clusters, and larger vesicles lacking internal content (Fig. 3C). High-resolution live-cell imaging of CESA7-containing organelles demonstrates the dynamic exchange of CSCs among the Golgi, SmaCCs, and the plasma membrane associated with microtubule bands.

Previous studies of primary cell wall CSCs showed that disruption of cortical microtubules did not affect the rate of insertion of CSCs into the plasma membrane (10), although CSC distribution over the membrane was transiently disorganized (9, 10). To test the effect of loss of microtubule bundles on secondary cell wall CSC insertion events, we measured the velocities and trajectories of CESA7-containing CSCs in the VND7::GR induction system after oryzalin treatment. In contrast to the dimethyl sulfoxide (DMSO) control (Fig. 4A), where the tracks of CSCs were restricted to the secondary cell wall bands, the plasma membrane-localized CSCs in oryzalin-treated cells were disorganized (Fig. 4A and movie S5). These CSC clusters were similar to the “swarms” of primary cell wall CSCs described after treatment with an intermediate (8) but not higher (18) concentration of oryzalin. In the absence of microtubule bands, the velocity of secondary cell wall CSCs at the plasma membrane was unaffected (313 ± 41 nm/min for control, 324 ± 33 nm/min for oryzalin-treated; $n = 12$ cells from 4 plants for each; Fig. 4B) while CSC insertion events continued (Fig. 4C and movie S6). Oryzalin treatment also did not affect the total cellulose content produced by the differentiating cells (fig. S4). In contrast, inhibiting cellulose biosynthesis with 2,6-dichlorobenzonitrile (DCB) treatment influenced only cellulose deposition, while microtubule formation was unaffected (fig. S5). Therefore, the banded microtubule pattern is independent from secondary cell wall formation, and although microtubules are important for the overall secondary cell wall banding pattern, they are not necessary for CSC delivery from endomembranes to the plasma membrane, nor for reaching peak CSC velocity.

We attribute the rapid formation of the secondary cell wall to both increased concentration and velocity of CSCs at the plasma membrane. Secondary cell wall CSCs are delivered to these domains by populations of Golgi-associated and independent SmaCCs. These insertion events are associated with, but do not require, microtubule bundles underlying secondary cell wall domains. These secondary cell walls provide structural support and the water-conducting functions necessary for plants to inhabit land. These data provide important insights into how land plants produce secondary cell walls with the ultrastructural features required for upright growth.

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support staff of the UBC Bioimaging Facility and R. White for statistical advice. The pTA7001 plasmid (VP16-GR vector) is available from N.-H. Chua of The Rockefeller University under a material transfer agreement; the VND7-GR line is available from T.D.; and the GFP::CESA3 and YFP::CESA7 VND7-GR lines are freely available from L.S. or S.D.M. for noncommercial purposes under a materials transfer agreement on the GVG system with N.-H. Chua. Further data are reported in the supplementary materials.

SUPPLEMENTARY MATERIALS

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Materials and Methods

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References (19–22)

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PLANT SCIENCE

Structure-function analysis identifies highly sensitive strigolactone receptors in *Striga*

Shigeo Toh,¹ Duncan Holbrook-Smith,¹ Peter J. Stogios,^{2,3} Olena Onopriyenko,² Shelley Lumba,¹ Yuichiro Tsuchiya,⁴ Alexei Savchenko,² Peter McCourt^{1*}

Strigolactones are naturally occurring signaling molecules that affect plant development, fungi-plant interactions, and parasitic plant infestations. We characterized the function of 11 strigolactone receptors from the parasitic plant *Striga hermonthica* using chemical and structural biology. We found a clade of polyspecific receptors, including one that is sensitive to picomolar concentrations of strigolactone. A crystal structure of a highly sensitive strigolactone receptor from *Striga* revealed a larger binding pocket than that of the *Arabidopsis* receptor, which could explain the increased range of strigolactone sensitivity. Thus, the sensitivity of *Striga* to strigolactones from host plants is driven by receptor sensitivity. By expressing strigolactone receptors in *Arabidopsis*, we developed a bioassay that can be used to identify chemicals and crops with altered strigolactone levels.

Strigolactones are small molecules that act as endogenous plant hormones to influence plant growth and development (1, 2). Unlike other plant hormones, multiple forms of bioactive strigolactones exist and vary in moieties attached to the A and B rings as well as the orientation of the D ring (fig. S1) (2). Aside from their hormonal role, strigolactones are also exuded through roots into the soil, where they act as exogenous signals to attract mycorrhizal fungi for a symbiotic interaction that brings nutrients to the plant (3). Unfortunately, parasitic plants of

the *Striga*, *Phelipanche*, and *Orobanchae* genera also use root-emitted strigolactones to locate a nearby host (2). Upon perception of strigolactones produced by host plants, seeds of parasitic plants germinate. Resulting seedlings attach themselves to the host to deplete them of nutrients with devastating consequences. In sub-Saharan Africa alone, *Striga* has infested up to two thirds of the arable land and represents the largest challenge to food security on that continent, affecting more than 100 million people in 25 countries (4). A mechanistic understanding of strigolactone synthesis and signaling particularly in *Striga* could open avenues for pest control.

Studies from model plants suggest that a family of related α/β hydrolases is central to strigolactone perception. For example, D14 (*DWARF14*) is a strigolactone receptor important for shoot branching in rice (5–7). A related α/β hydrolase gene, *HYPOSENSITIVE TO LIGHT/KARRIKIN INSENSITIVE2* (*HTL/KAI2*), herein designated *HTL*, has been shown to play a role in *Arabidopsis* germination (8). Because *Striga* requires strigolac-

tones to germinate, by inference *HTL* homologs may have similar roles in parasitic plant species. *Arabidopsis* *HTL*, however, binds a smoke-derived germination stimulant, karrikin (9). In contrast, *Striga* does not germinate in response to karrikin but is sensitive to picomolar concentrations of strigolactones (10). Moreover, *Striga* appears to differentiate among hosts by sensing different combinations of strigolactones (10). Because *Striga hermonthica* (*Striga*) is an obligate and outcrossing hemiparasite, the functional roles of *Striga* *HTL/KAI2* homologs (*ShHTLs*) in strigolactone perception are difficult to elucidate. Here, we describe the roles of *ShHTLs* in strigolactone perception.

Phylogenetic analysis of public databases led us to identify a nested clade of 11 *HTL* homologs in *Striga* (Fig. 1A). To analyze the function of these *ShHTL* genes, we expressed each of the 11 *ShHTLs* individually in an *Arabidopsis* loss-of-function *HTL/KAI2* mutant (*htl-3*) background and assayed for germination phenotypes. Seeds of the *Arabidopsis* ecotype Columbia germinate poorly after exposure to high temperature, but this thermoinhibition is alleviated by strigolactone addition (11). The strigolactone-dependent rescue of thermoinhibited seeds requires a functional *AtHTL* gene, which provided the basis for introducing *ShHTL* genes into an *htl-3* genetic background. We monitored the functional activity of these genes by assaying for suppression of thermoinhibited seeds. Thermoinhibited *htl-3* seed constitutively overexpressing *AtHTL* driven by the 35S promoter germinated upon addition of a synthetic strigolactone, GR24 (fig. S2). Similarly, thermoinhibited *htl-3* seed containing different *ShHTL* transgenes germinated on GR24 to varying degrees, depending on the transgene (fig. S2). To compare lines, we determined the effective concentration of GR24 required for 50% germination (EC_{50}) of transgenic seed (fig. S3). The EC_{50} of a transgenic line overexpressing *AtHTL* is lower than that of the wild type, indicating increased strigolactone responsiveness (Fig. 1B). In contrast, *ShHTL1*, *ShHTL2*, and *ShHTL3* expression lines were less responsive to GR24 compared with either wild type or misexpressed *AtHTL* seed (Fig. 1B). *ShHTL8* and *ShHTL9* transgenics were less

¹Cell and Systems Biology, University of Toronto, 25 Wilcocks Street, Toronto M5S 3B2, Canada. ²Department of Chemical Engineering and Applied Chemistry, Banting and Best Department of Medical Research, University of Toronto, 200 College Street, Toronto M5S 3E5, Canada. ³Center for Structural Genomics of Infectious Diseases, contracted by National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. ⁴Institute of Transformative Bio-Molecules, Nagoya University, Japan, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8602, Japan.
*Corresponding author. E-mail: peter.mccourt@utoronto.ca

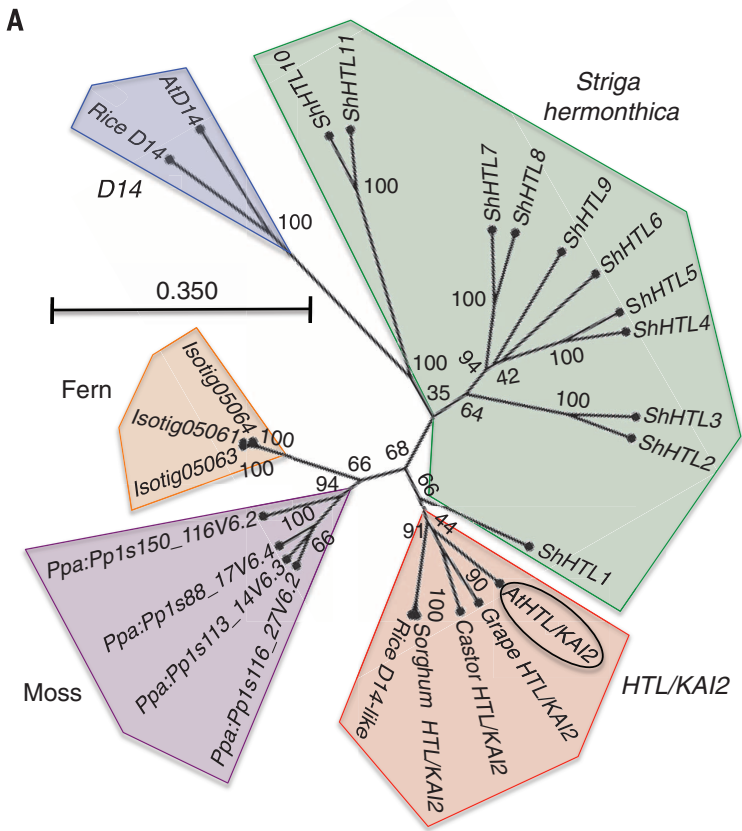
effective, whereas *ShHTL4*, *ShHTL5*, and *ShHTL6* were more responsive compared with *AtHTL* (Fig. 1B). Complementation results from a study in which four *ShHTL* genes were transformed

into an *Arabidopsis htl* mutant were consistent with our findings (12). *ShHTL7* transgenic seed were four to five orders of magnitude more responsive than other lines (Fig. 1C). This pico-

molar level of sensitivity is in the realm of *Striga* strigolactone sensitivity, which was unexpected because the remainder of the strigolactone signaling machinery in our transgenic plants is

Fig. 1. *Striga* *ShHTL* genes.

(A) Phylogenetic tree of *ShHTL* genes from *Striga hermonthica* and a variety of nonparasitic plants. The circle represents *Arabidopsis HTL/KAI2*. Bootstrap values of 100 replicates are indicated by each node. (B) EC₅₀ of GR24^{rac} that germinates 50% of *htl-3* seeds containing an *AtHTL* or *ShHTL* transgene. Numbers represent biological replicates from three independent transformants. EC₅₀ was calculated from fig. S3. (C) Representative picture of thermoinhibited wild-type or *htl-3* 35S::*ShHTL7* seed exposed to different concentrations of GR24^{rac}.



B

	EC ₅₀ (μM)
wild type	0.90
<i>htl-3</i>	
<i>AtHTL</i>	0.05
<i>ShHTL1</i>	8.79
<i>ShHTL2</i>	3.43
<i>ShHTL3</i>	10.91
<i>ShHTL4</i>	0.01
<i>ShHTL5</i>	0.01
<i>ShHTL6</i>	0.03
<i>ShHTL7</i>	17.5 x 10 ⁻⁶
<i>ShHTL8</i>	0.10
<i>ShHTL9</i>	0.15

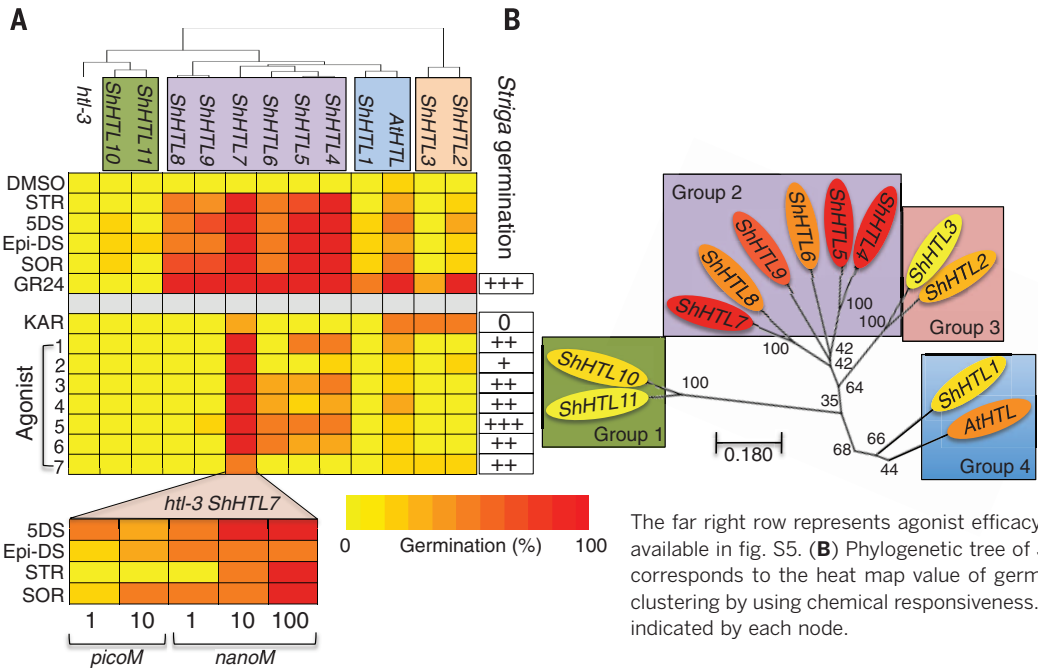
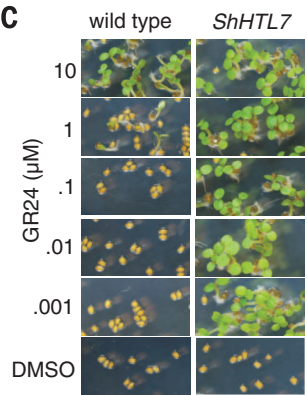


Fig. 2. Chemical responsiveness of *ShHTL* transgenics. (A) Germination rate of *htl-3*, *AtHTL*, and *ShHTL* seed on strigolactones (1 μM) and strigolactone agonists (10 μM). (Inset) An expansion of strigolactone concentrations tested on *ShHTL7* seed. Genes were clustered on the basis of their similarity of response to all chemicals. Strigol (STR), 5-deoxy strigol (5DS), 2'-epi-5-deoxy strigol (Epi-DS), and Sorgolactone (SOR) experiments were performed by using the thermotolerance assay. Karrikin1 (KAR) and agonists 1 to 7 experiments were performed by using the primary dormancy assay. Details are available in figs. S2 and S4.

encoded by the *Arabidopsis* genome. Thus, it appears that different *ShHTL* hydrolases are sufficient to confer the extreme sensitivity of parasitic plants to strigolactones.

Although the introduction of different *ShHTLs* into *htl-3* resulted in variable GR24 seed responsiveness, this strigolactone is synthetic and exists in two isomeric forms (GR24^{rac}). To test the contribution of each *ShHTL* more rigorously, we analyzed the response of our transgenics using a collection of naturally occurring strigolactones, which are all capable of germinating *Striga*. Transgenics expressing *ShHTL4* to *ShHTL9* were the most sensitive to natural strigolactones (Fig. 2A). This group of genes, group 2, defines a subclade within the *ShHTL* phylogeny that arose from a common ancestor (Fig. 2B). *ShHTL7* seed was the most responsive because it germinated at picomolar concentra-

tions for 5DS, 2'-Epi-5DS, and sorgolactones but responded to strigol in the nanomolar range (Fig. 2A). This suggests that the presence of a hydroxyl group on carbon 5 of the A-ring of strigol decreases binding to this receptor and may explain why crop cultivars producing high levels of 5DS that lack this hydroxyl group are so susceptible to infection (10). Last, two *ShHTL* lines (*ShHTL2* and *ShHTL3*) responded to karrikin, although this butenolide does not germinate *Striga* seed (Fig. 2A). This suggests that activation of these receptors is not sufficient for *Striga* germination. Overall, our functional results correlate well with enzymatic activities of *ShHTLs* on 5DS with the exception of *ShHTL10* and *ShHTL11* (13). Although *ShHTL10* and *ShHTL11* were biochemically active, they could not suppress the *htl* mutant phenotype, suggesting a different role for these *ShHTLs* other than germination.

We then probed the promiscuity of *ShHTL* receptors using a collection of synthetic strigolactone agonists that show different potencies for germinating *Striga* (Fig. 2A and fig. S4) (14). In this experiment, we assayed germination of underripened seed. This dormancy assay, similar to thermoinhibition, is alleviated by strigolactone and requires a functional *HTL* gene (11). Within group 2, we found that *ShHTL4* to *ShHTL7*—but not *ShHTL8* and *ShHTL9*—showed the broadest range of sensitivity to various agonists. Of the 11 strigolactone receptors in *Striga*, it appears that *ShHTL4* to *ShHTL7* form a minimal set of receptors sufficient for *Striga* germination. Clustering analysis based on germination efficiencies on natural strigolactones and synthetic agonists confirmed the formation of the *ShHTL4* to *ShHTL7* subclade and identified *ShHTL7* as the most functionally sensitive receptor

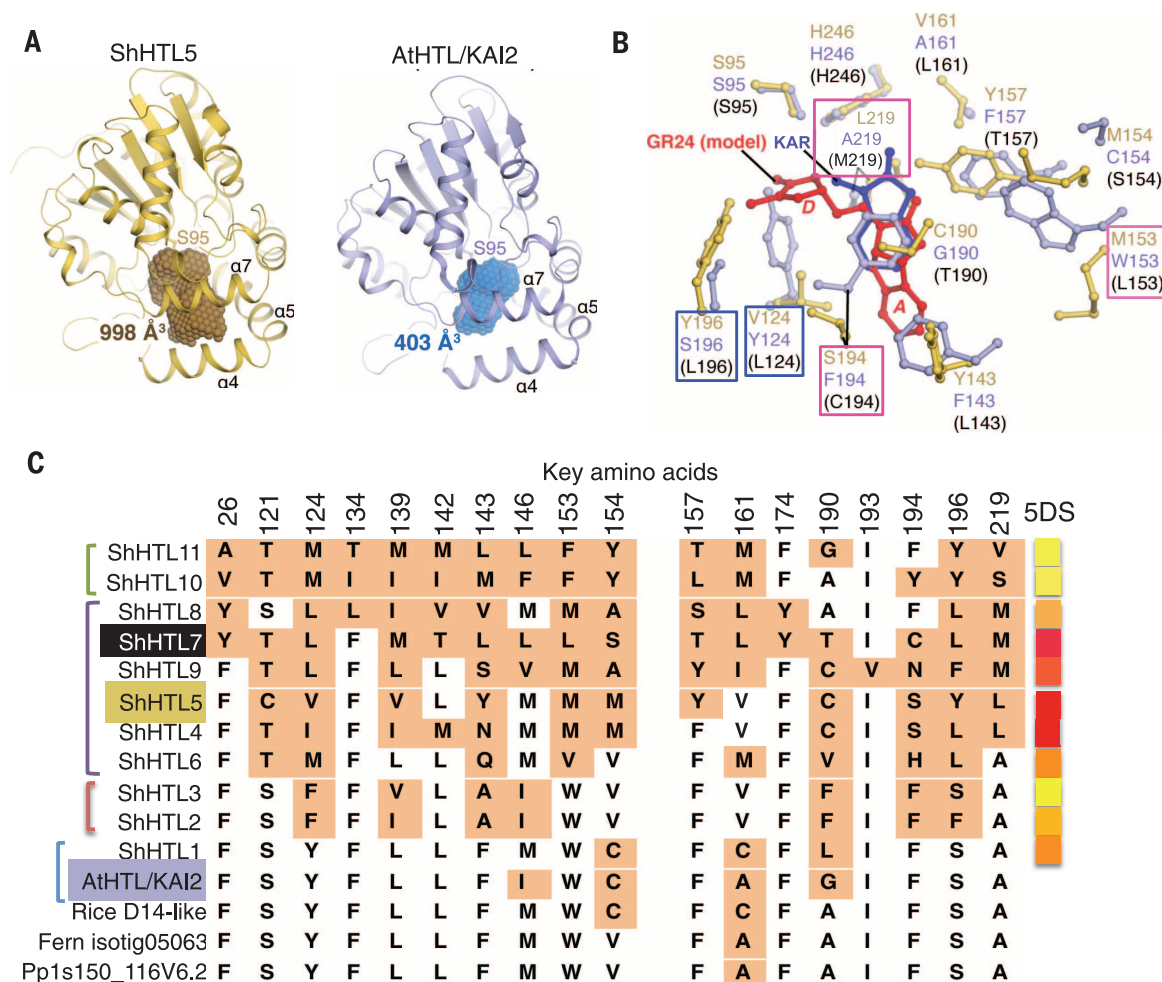


Fig. 3. Structural analysis of ShHTL5. (A) Structures of ShHTL5 and AtHTL (PDB 4JYM). Spheres indicate solvent accessible volume comprising active site cavities. (B) Comparison of active site residues of strigolactone receptors. Sticks are shown and labeled for active site residues showing notable residue substitutions between ShHTL5 (yellow) and AtHTL (blue) plus the catalytic serine and histidine residues; equivalent ShHTL7 residues are labeled in black. Karrikin1 (KAR, blue) bound to AtHTL (PDB 4JYM) and GR24 (red; A and D rings of GR24 are labeled) in silico docked into the active sites of ShHTL5.

Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (C) Sequence alignment of solvent-exposed active site amino acid residues for ShHTL proteins. Amino acids that differ from AtHTL are colored orange. AtHTL is blue, ShHTL5 is yellow, and ShHTL7 is black. A fully expanded alignment can be found in fig. S6. The far right column is a heat map of germination of each transgenic in response to 5DS from Fig. 2A. Color brackets represent groups based on Fig. 2B.

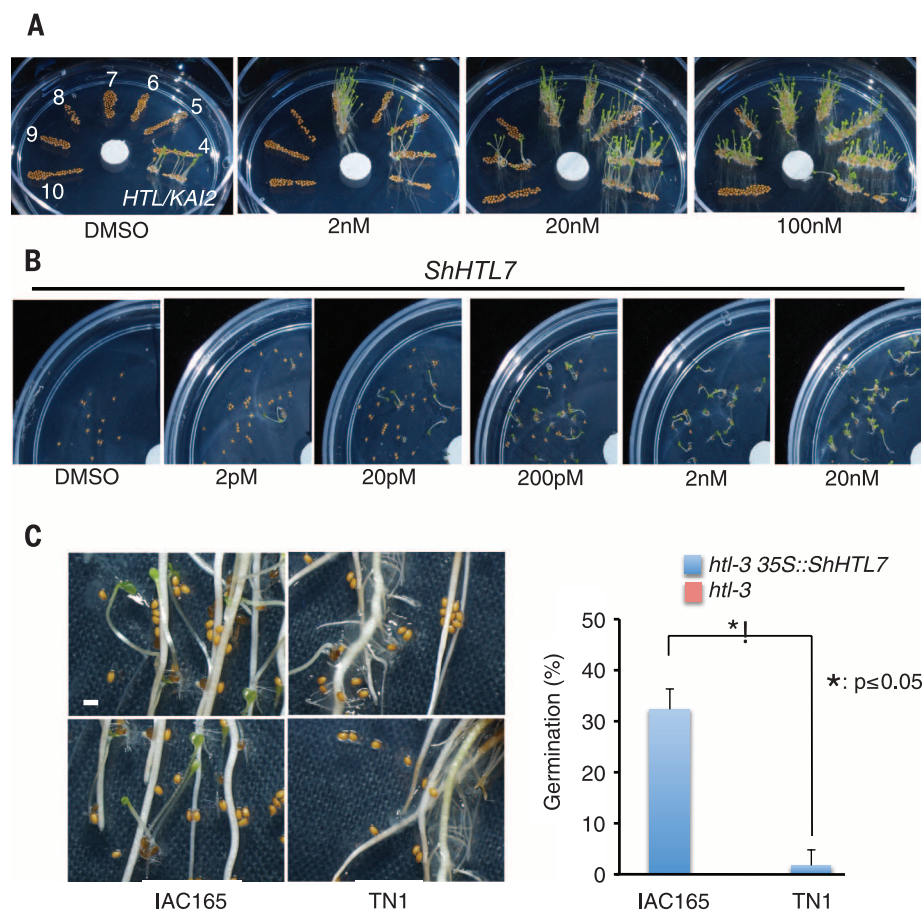


Fig. 4. Strigolactone bioassay. (A) Germination of thermoinhibited *htl-3* seed containing different *ShHTL* transgenes (*ShHTL4* to *ShHTL10*). The center filter disc was impregnated with GR24^{rac}, and black labels are the calculated final concentrations of GR24^{rac} in the plate. (B) Germination of transgenic *htl-3* seed containing *ShHTL7* on varying concentrations of GR24^{rac}. The center filter disc was impregnated with GR24^{rac}, and black labels are as in (A). (C) Germination of thermoinhibited *htl-3* 35S::*ShHTL7* in the presence of a high- (IAC165) or low- (TN1) strigolactone-emitting rice cultivars. (Left) Representative picture of *Arabidopsis htl-3* 35S::*ShHTL7* seeds sprinkled around rice roots. (Right) Quantification of three independent experiments $\pm$ SD. Scale bar, 1 mm.

(Fig. 2A). Although the phylogenetic tree based on receptor sequence is mostly reflected by clustering according to functional response, the use of natural strigolactones and synthetic agonists increases the functional resolution within a clade of receptors (Fig. 2B). Thus, chemical probes can be used to dissect functions of individual *Striga* receptors not obvious through genetic or evolutionary studies. Our results indicate that this set of four receptors should be the focus of chemical screens in the development of compounds to combat *Striga*.

To establish the molecular basis for strigolactone sensitivity by *ShHTL* proteins, we determined and characterized the crystal structure of *ShHTL5* at a resolution of 2.48 Å because *ShHTL5* belongs to the most sensitive group of receptors (group 2) (table S1). Structural conservation between *ShHTL5* and *AtHTL* [Protein Data Bank (PDB) 4JYM, PMID 23613584] structures (root mean square deviation of 1.0 Å over 268 matching Ca atoms) allowed comparative analysis of their

active sites (Fig. 3A). In both *ShHTL5* and *AtHTL*, the active site is localized to a cavity formed by respective $\alpha 4$, $\alpha 5$, and $\alpha 7$ helices (Fig. 3A), which was more than twice as large for *ShHTL5* as compared with *AtHTL* (998 versus 403 Å³) (Fig. 3A). There is also substantial variation among residues localized to the active site cavity, with 9 out of 18 *ShHTL5* residues showing deviation from their counterparts in *AtHTL* (Fig. 3B). Particularly, the substitutions of Y124 and S196 in *AtHTL* to V124 and Y196, respectively, in *ShHTL5* deepened the active site cleft in the latter protein, making it more complementary to the D-ring of the GR24 molecule (Fig. 3B, blue boxes). In contrast, the Y124 in *AtHTL* would occlude this region of GR24. Other differences in active site composition included alteration at positions (W153, F194, and A219) involved in positioning the karrikin ligand in *AtHTL* but that are represented as M153, S194, and L219, respectively, in *ShHTL5* (Fig. 3B, pink boxes) (9). These structural differences provide a substantially different

chemical environment in the *ShHTL5* active site, suggesting tailoring of the active site to recognize the strigolactone scaffold and discriminate against karrikin.

Taking advantage of the structural characterization of *ShHTL5*, an analysis of the variation among active-site residues across the *ShHTL* protein family rationalized the enhanced response of *ShHTL7*- versus *ShHTL5*-harboring transgenic plants to strigolactones (Fig. 3B). Structural analyses revealed that the active-site pocket of *ShHTL1* was most similar to that of *AtHTL* (14 identical and one chemically similar active-site residues); the *ShHTL7* active site was most distinct (two identical and five chemically similar active-site residues); and the *ShHTL5* active site was intermediary (four identical and five chemically similar residues). The degree of similarity of *ShHTL* active sites to those of *AtHTL* is consistent with low, intermediate, and high response of *ShHTL1*-, *ShHTL5*-, and *ShHTL7*-expressing transgenic plants to GR24 (Fig. 3C). This observation prompted us to (Fig. 4, A and B) hypothesize that *ShHTLs* have evolved differential strigolactone-binding affinities through modulation of their active site architecture.

Success in controlling *Striga* infestations will require integrated strategies involving both chemical control and plant breeding (15, 16). *Striga* itself is difficult to study and, as a noxious weed, is generally inaccessible to most researchers. *Arabidopsis* seed expressing *ShHTL7* transgenic seed could function as a bioassay for strigolactone. Indeed, thermoinhibited *ShHTL7* seed responded to GR24 concentrations as low as 2 pM. High and low germination rates of *ShHTL7* seeds sprinkled on rice roots reflected cultivars emitting high (IAC165) and low levels (TN1) of strigolactone, respectively (Fig. 4C) (16).

Striga receptors possess larger and modified active site architectures with high sensitivities to strigolactone compared with that of *Arabidopsis* HTL. We hypothesize that these changes have contributed to the ability of *Striga* to expand its host range through recognition of a wide range of strigolactones (12). Although broad specificity and high sensitivity are not generally associated, biochemical studies involving soluble multidrug transporters demonstrate that the evolution of large binding pockets to accommodate multiple ligands does not necessarily result in a loss of sensitivity (17). Multidrug transporters possess broad specificity because of large hydrophobic binding pockets but also form high-affinity interactions through hydrophobic and electrostatic interactions. It will be interesting to determine whether *Striga hermonthica* strigolactone receptors have similar biochemical mechanisms.

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GenBank under accession KR013120 to KR013131. S.T., D.H.-S., P.M., and the University of Toronto have filed a U.S. provisional patent application (US62/151,701) that relates to the specific topic “Strigolactone biosensors.” We declare no financial conflicts of interest in relation to the work. The supplementary materials contain additional data.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S6

Table S1

References (18–30)

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ONCOLOGY

Genomic correlates of response to CTLA-4 blockade in metastatic melanoma

Eliezer M. Van Allen,^{1,2,3*} Diana Miao,^{1,2*} Bastian Schilling,^{4,5*} Sachet A. Shukla,^{1,2} Christian Blank,⁶ Lisa Zimmer,^{4,5} Antje Sucker,^{4,5} Uwe Hillen,^{4,5} Marnix H. Geukes Foppen,⁶ Simone M. Goldinger,⁷ Jochen Utikal,^{5,8,9} Jessica C. Hassel,¹⁰ Benjamin Weide,¹¹ Katharina C. Kaehler,¹² Carmen Loquai,¹³ Peter Mohr,¹⁴ Ralf Gutzmer,¹⁵ Reinhard Dummer,⁷ Stacey Gabriel,² Catherine J. Wu,^{1,2} Dirk Schadendorf,^{4,5,†} Levi A. Garraway^{1,2,3,†}

Monoclonal antibodies directed against cytotoxic T lymphocyte–associated antigen-4 (CTLA-4), such as ipilimumab, yield considerable clinical benefit for patients with metastatic melanoma by inhibiting immune checkpoint activity, but clinical predictors of response to these therapies remain incompletely characterized. To investigate the roles of tumor-specific neoantigens and alterations in the tumor microenvironment in the response to ipilimumab, we analyzed whole exomes from pretreatment melanoma tumor biopsies and matching germline tissue samples from 110 patients. For 40 of these patients, we also obtained and analyzed transcriptome data from the pretreatment tumor samples. Overall mutational load, neoantigen load, and expression of cytolytic markers in the immune microenvironment were significantly associated with clinical benefit. However, no recurrent neoantigen peptide sequences predicted responder patient populations. Thus, detailed integrated molecular characterization of large patient cohorts may be needed to identify robust determinants of response and resistance to immune checkpoint inhibitors.

Blockade of cytotoxic T lymphocyte antigen-4 (CTLA-4), an inhibitor of T cell activation, with the monoclonal antibody ipilimumab yields improvements in overall survival in patients with metastatic melanoma as a monotherapy (1, 2) or in combination with other T cell immune checkpoint inhibitors (3, 4). Although overall single-agent response rates are low, a long-term clinical benefit is consistently observed for ~20% of treated patients (5, 6). Preclinical and clinical studies have suggested that tumor-specific missense mutations may generate individual neoantigens that mediate response to ipilimumab and other immune checkpoint inhibitors (7–10). Clinical studies of exceptional responders (11) and of small cohorts of melanoma patients have highlighted *NRAS* mutation status, total neoantigen load, and a neoantigen-derived tetrapeptide signature as possible correlates of response to ipilimumab in

metastatic melanoma (12, 13). RNA-based studies have also identified gene expression signatures linked to immune infiltration within the tumor microenvironment that correlate with overall survival, neoantigen load (14, 15), and resistance to immunotherapy (16). To date, however, comprehensive genomic studies of tumor- and immune-related factors in larger (i.e., >100 patients) clinical cohorts have not been reported.

We hypothesized that both tumor-specific neoantigens and the tumor immune microenvironment might influence clinical benefit from ipilimumab. To test this, we performed whole-exome sequencing (WES) on a cohort of 110 patients with metastatic melanoma from whom pretreatment tumor biopsies were available for study (Fig. 1A). Tumor whole-transcriptome sequencing was performed in 42 of these patients, of whom 40 had matched WES. This cohort included 92 cutaneous, 4 mucosal, and 14 occult

melanomas. After WES of matched tumor and germline samples (17), quality-control metrics were applied to ensure sensitive mutation detection (18). Average exome-wide target coverage was 183.7-fold for tumor samples and 157.2-fold for germline samples. We performed somatic mutation identification (table S1) and germline human lymphocyte antigen (HLA) typing (table S2) using established methods (14, 19). The median non-synonymous mutational load was 197 per sample (range: 7 to 5854), which is consistent with the known high mutational loads in cutaneous melanoma (13, 20).

To stratify our cohort, “clinical benefit” was defined using a composite end point of complete response or partial response to ipilimumab by RECIST criteria (21) or stable disease by RECIST criteria with overall survival greater than 1 year ($n = 27$). “No clinical benefit” was defined as progressive disease by RECIST criteria or stable disease with overall survival less than 1 year ($n = 73$). The basis for these designations stems from clinical trials demonstrating that ipilimumab significantly improves median overall survival, with a subset of patients surviving beyond 2 years (~20%), but does not affect progression-free survival

¹Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02215, USA. ²Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA. ³Center for Cancer Precision Medicine, Dana-Farber Cancer Institute, Boston, MA 02215, USA. ⁴Department of Dermatology, University Hospital, University Duisburg–Essen, 45147 Essen, Germany. ⁵German Cancer Consortium (DKTK), 69121 Heidelberg, Germany. ⁶Department of Medical Oncology, Netherlands Cancer Institute, 1066 CX Amsterdam, Netherlands. ⁷Department of Dermatology, University Hospital Zurich, 8091 Zurich, Switzerland. ⁸Skin Cancer Unit, German Cancer Research Center (DKFZ), 69121 Heidelberg, Germany. ⁹Department of Dermatology, Venerology, and Allergy, University Medical Center, Ruprecht-Karls University of Heidelberg, 68167 Mannheim, Germany. ¹⁰Department of Dermatology, University Hospital, Ruprecht-Karls University of Heidelberg, 69120 Heidelberg, Germany. ¹¹Department of Dermatology, University Hospital Tübingen, 72076 Tübingen, Germany. ¹²Department of Dermatology, University Hospital Kiel, 24105 Kiel, Germany. ¹³Department of Dermatology, University Medical Center Mainz, 55131 Mainz, Germany. ¹⁴Department of Dermatology, Elbe-Kliniken, 21614 Buxtehude, Germany. ¹⁵Department of Dermatology and Allergy, Skin Cancer Center Hannover, Hannover Medical School, 30625 Hannover, Germany. *These authors contributed equally to this work. †Corresponding author. E-mail: levi_garraway@dfci.harvard.edu (L.A.G.); dirk.schadendorf@uk-essen.de (D.S.)

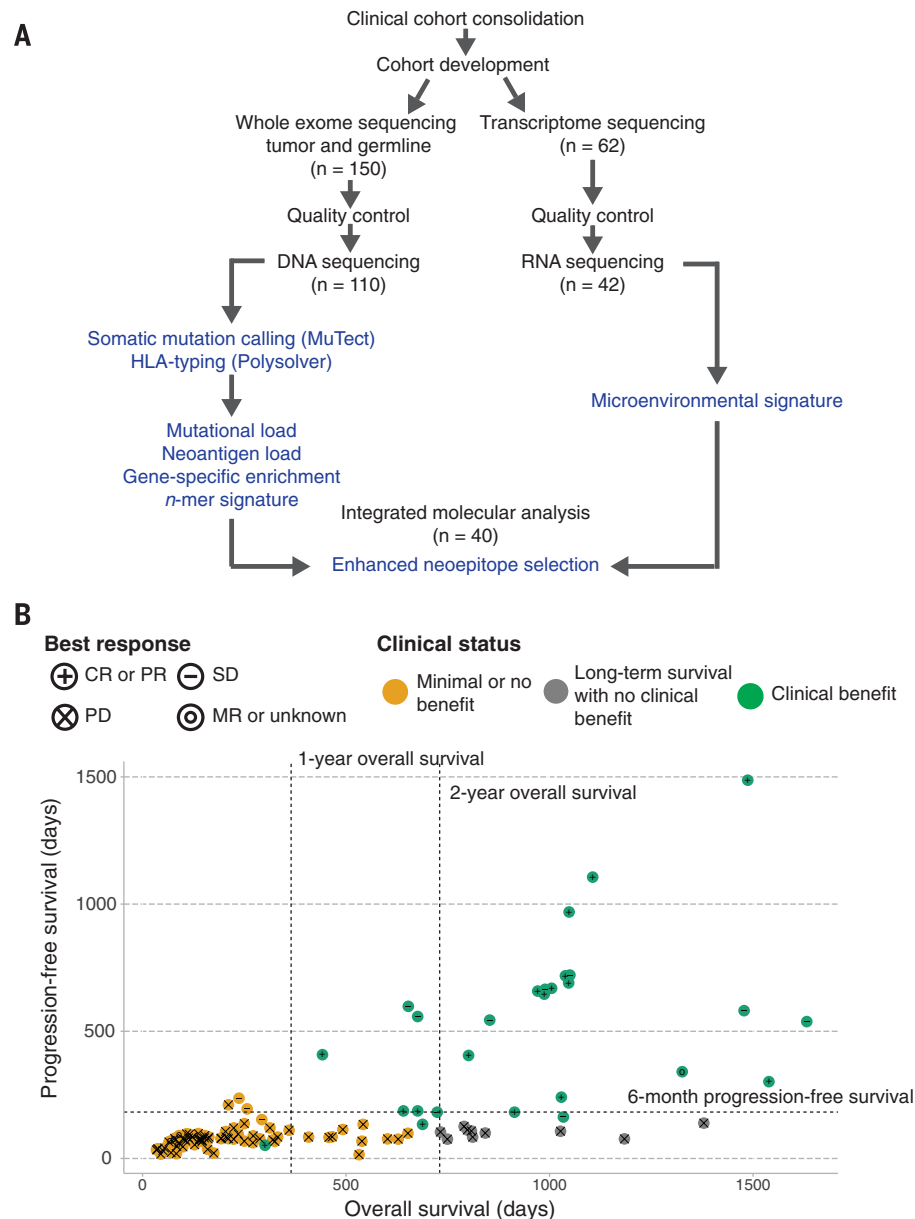


Fig. 1. Study design and clinical stratification. (A) Patients ($n = 150$) were identified for whole-exome sequencing of tumor and germline DNA. To be included in the original clinical cohort, patients had to have received ipilimumab monotherapy for metastatic cutaneous melanoma, have pretreatment germline and tumor samples available for sequencing, and have had overall survival for >14 days after initiation of ipilimumab therapy. Of these patients, 110 were eventually included in analysis after exclusions due to inadequate postsequencing quality control ($n = 40$) (18). Manual review of raw sequencing data was performed to exclude samples with evidence suggesting low purity, high contamination by ContEst (33), or discordant copy number quality control. Of the patients, 62, including 2 who failed DNA quality-control, had FFPE tumor samples available for transcriptome sequencing. After manual review for quality control following RNA sequencing, 42 samples were also analyzed for tumor microenvironment signatures, and 40 with matched WES were analyzed for neoantigen expression (14). **(B)** Patients were stratified into response groups based on RECIST criteria (21) (CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; MR, mixed response); duration of overall survival (OS); and duration of progression-free survival (PFS). All two-way comparisons were done comparing patients who achieved clinical benefit with ipilimumab (CR or PR by RECIST criteria or OS >1 year with SD by RECIST criteria) ($n = 27$) to those with minimal or no benefit from ipilimumab (PD by RECIST criteria or OS <1 year with SD by RECIST criteria) ($n = 73$). An additional cohort of patients who achieved long-term survival (OS >2 years) after ipilimumab treatment with early tumor progression (PFS <6 months) were considered separately ($n = 10$).

(PFS) (5, 22). A separate group of 10 patients showed early progression on ipilimumab (PFS of <6 months), but their overall survival patterns exceeded 2 years; these patients were considered as a separate patient subgroup (Fig. 1B and tables S2 and S3).

Overall, nonsynonymous mutational load was significantly associated with clinical benefit from ipilimumab ($P = 0.0076$; Mann-Whitney test) (Fig. 2A). This result confirms previous findings for ipilimumab in melanoma (13) and is consistent with observations regarding response to other immune checkpoint inhibitors in cancer (23, 24). Clinical metrics—such as patient age, gender, tumor histology, primary tumor site, number of therapies received before ipilimumab, and lactate dehydrogenase levels at initiation of ipilimumab monotherapy—showed no significant correlation with clinical response to ipilimumab in this cohort ($P > 0.05$ for all) (table S3). The subset of patients that showed long-term survival tracked with the subset that showed no clinical benefit, in terms of mutational load, but the sample size in this group was too small to draw definitive conclusions (Fig. 2A). No genes were enriched for nonsynonymous mutations, including *BRAF* and *NRAS*, in the patient subgroups that had clinical benefit or no clinical benefit (figs. S1 and S2).

Next, we sought to determine the relation between neoantigen load and clinical benefit to ipilimumab. First, we identified putative immunogenic 9- and 10-amino acid neoantigens with ≤ 500 nM binding affinity for HLA class I molecules across the cohort, using patient-specific nonsynonymous mutations and HLA types (tables S2 and S4) (14). The median predicted neoantigen load was 369 neoantigens per sample (range: 9 to 14,880). Overall, neoantigen load was significantly associated with clinical benefit ($P = 0.027$; Mann-Whitney) (Fig. 2A), although high neoantigen load outliers among nonresponders and low neoantigen load outliers among responders were observed. Neoantigen load was strongly correlated with mutational load (Spearman's $\rho = 0.97$, $P < 0.0001$) as expected, given that neoantigens often arise from nonsynonymous mutations. The association between neoantigen load and clinical benefit remained significant when randomly redistributed HLA types were used to infer putative neoantigen binders ($P = 0.0096$; Mann-Whitney) (fig. S3).

We then sought to determine the association between aggregate neoantigen properties and clinical benefit. The correlation between neoantigen load and clinical benefit diminished when we applied increasingly stringent thresholds for affinity of binding ($P = 0.034$, 0.039, and 0.042 for affinity thresholds of <250 nM, 100 nM, and 50 nM, respectively; Mann-Whitney) (Fig. 2B). We also applied multivariate models that controlled for prior RAF inhibitor treatment and M class (metastasis) at start of therapy (18). These analyses confirmed that patients with high neoantigen or mutational loads (>100) were significantly more likely to have clinical benefit from ipilimumab ($P = 0.0371$ and $P = 0.0169$, respectively).

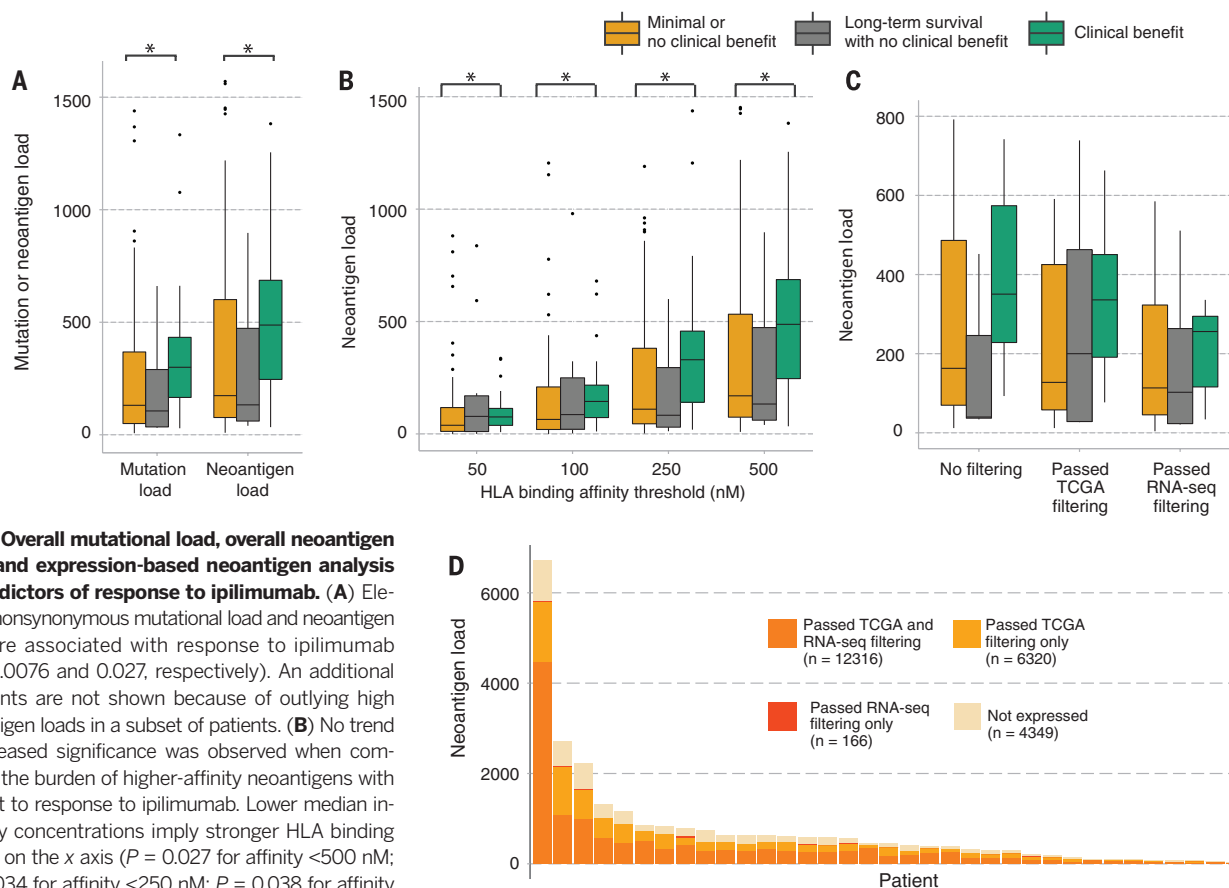


Fig. 2. Overall mutational load, overall neoantigen load, and expression-based neoantigen analysis as predictors of response to ipilimumab. (A) Elevated nonsynonymous mutational load and neoantigen load are associated with response to ipilimumab ($P = 0.0076$ and 0.027 , respectively). An additional 20 points are not shown because of outlying high neoantigen loads in a subset of patients. (B) No trend in increased significance was observed when comparing the burden of higher-affinity neoantigens with respect to response to ipilimumab. Lower median inhibitory concentrations imply stronger HLA binding affinity on the x axis ($P = 0.027$ for affinity <500 nM; $P = 0.034$ for affinity <250 nM; $P = 0.038$ for affinity <100 nM; $P = 0.042$ for affinity <50 nM). An additional 34 points are not shown because of outlying high neoantigen loads in a subset of patients. (C) A sample size of 40 patients with complete DNA-sequencing, RNA-sequencing, and clinical annotation was insufficient to discern significant differences in neoantigen load or expressed neoantigen load among response cohorts, but a trend was observed for increased neoantigen load among patients with clinical benefit compared with those with no clinical benefit ($P > 0.05$ for all). (D) Patient-specific RNA-sequencing provides distinct information on tumor gene expression compared with TCGA melanoma data from a separate patient cohort. Although TCGA and RNA-seq data agree on the expression of the majority of neoantigens ($n = 12,316$) for 40 patients who had high-quality DNA- and RNA-sequencing data available for neoantigen and gene expression analysis, TCGA expression data overestimate the number of neoantigens expressed by 6320 in this patient cohort, and 166 neoantigens that are expressed by patient tumors would be missed by TCGA filtering alone. Additionally, a large proportion of neoantigens ($n = 4349$) are expressed at negligible levels in patient tumors. Asterisks (*) indicate $P < 0.05$.

We then investigated whether any recurrent neoantigens or neoantigen epitopes might predict ipilimumab response. Of the 75,179 unique neoantigens in our cohort, 28 (~0.04%) were found in more than one patient having a clinical benefit but were absent in all patients having no clinical benefit or long-term survival (Table 1). Examination of these recurrent neoantigens did not reveal any shared features or features exclusive to responders. Previously described immunogenic nonamers identified in patients who achieved clinical benefit from immune checkpoint blockade were not observed in any patient within this cohort, including several that have undergone experimental validation (7, 11, 13, 23). Furthermore, a tetrapeptide signature (13) previously associated with ipilimumab response was not enriched in the cohort with clinical benefit relative to that with no clinical benefit (fig. S4) (25). Thus, the vast majority of clinical benefit-associated neoantigens and neoantigen epitopes identified through DNA sequencing appear to be private events without obvious recurrent features.

To evaluate neoantigens most likely to engender an immune response, we next examined whether predicted immunogenic neoantigens were expressed in mutated tumors. Here, we leveraged paired DNA- and RNA-sequencing data obtained for 40 patients (13 with clinical benefit, 22 with no clinical benefit, and 5 showing long-term survival) from whom high-quality archival formalin-fixed, paraffin-embedded (FFPE) tumor tissue was available (18). Among these patients, the median number of predicted neoantigens using nonsynonymous mutations and HLA typing alone was 395 per patient (range: 12 to 6984). Filtering these putative neoantigens by using patient-matched transcriptome data decreased the median neoantigen load to 198 per patient (range: 4 to 4622). A similar filtering approach that used unmatched melanoma gene expression data from The Cancer Genome Atlas (TCGA) (18, 26) also eliminated neoantigens unlikely to be expressed (in this case, the median neoantigen load was 347 per patient; range: 12 to 6159); however, the remaining neoantigens only partially overlapped

those that were inferred using patient-matched RNA (Fig. 2, C and D).

Conceivably, an inadequate immune response to tumor neoantigens could also be explained by aberrant HLA class I expression, which has been previously observed in melanoma (27). However, we found that HLA class I genes were expressed in all patients and in similar amounts across response groups (fig. S5, A and B). Moreover, somatic mutations in HLA class I genes were rare in this cohort and did not segregate by response group (table S5). Therefore, matched genome and transcriptome sequencing appeared to improve the identification of patient-specific neoantigens, which were not impacted by aberrant HLA expression or mutation status.

Finally, we leveraged the total transcriptome data from this cohort (42 patients total) to characterize an RNA expression profile in the tumor microenvironment previously associated with immune infiltrate. Specifically, the geometric mean of expression of granzyme A (*GZMA*) and perforin (*PRFI*) has been shown to correlate with

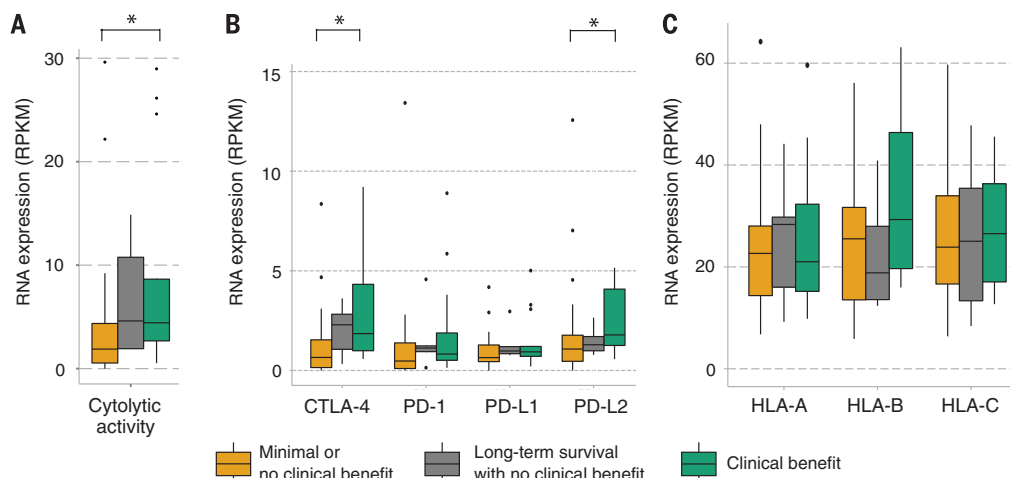
Table 1. Recurrent neoantigens identified exclusively in the cohort showing clinical benefit with their associated HLA types. Variants were manually reviewed in the Integrated Genomics Viewer (34).									
	Patient	Gene	HLA	Neoantigen		Patient	Gene	HLA	Neoantigen
1	Pat117	BMPER	A11:01	CIKTCDNWNK	13	Pat117	FAM5B	A02:01	FQDSALLQLI
	Pat123	BMPER	A31:01	CIKTCDNWNK		Pat117	FAM5B	C02:02	FQDSALLQLI
2	Pat174	CGB8	C02:02	SSSKAPLPSL		Pat123	FAM5B	C02:02	FQDSALLQLI
	Pat88	CGB2	C16:01	SSSKAPLPSL		Pat123	FAM5B	C04:01	FQDSALLQLI
3	Pat132	CLCN4	C04:01	FFATLVAAF	14	Pat117	FAM5B	C02:02	YTQGFQDSAL
	Pat132	CLCN4	A23:01	FFATLVAAF		Pat123	FAM5B	C02:02	YTQGFQDSAL
	Pat38	CLCN4	C02:02	FFATLVAAF	15	Pat21	FAM83B	B08:01	YARSCVPSL
	Pat132	CLCN4	C07:01	SFFATLVAAF		Pat21	FAM83B	C07:01	YARSCVPSL
	Pat132	CLCN4	A23:01	SFFATLVAAF		Pat88	FAM83B	C16:01	YARSCVPSL
	Pat38	CLCN4	C07:01	SFFATLVAAF		Pat88	FAM83B	B14:02	YARSCVPSL
5	Pat132	CLCN4	C07:01	ATLVAAFTL	16	Pat21	FAM83B	C07:01	YARSCVPSLF
	Pat138	CLCN4	B15:17	ATLVAAFTL		Pat88	FAM83B	C16:01	YARSCVPSLF
	Pat38	CLCN4	C07:01	ATLVAAFTL	17	Pat105	HSF5	A02:01	FVIGTEQAV
	Pat132	CLCN4	B08:01	TLWRSFFATL		Pat174	HSF5	A02:01	FVIGTEQAV
	Pat132	CLCN4	A23:01	TLWRSFFATL	18	Pat105	HSF5	C05:01	GSDIMSFVI
	Pat38	CLCN4	A02:01	TLWRSFFATL		Pat174	HSF5	C02:02	GSDIMSFVI
7	Pat07	CNTNAP5	A01:01	FSADIFFFF	19	Pat138	LATS2	A32:01	SLVETPNYI
	Pat07	CNTNAP5	C07:02	FSADIFFFF		Pat38	LATS2	A02:01	SLVETPNYI
	Pat07	CNTNAP5	C07:01	FSADIFFFF	20	Pat132	LOX	A01:01	HTQGLSPDCY
	Pat77	CNTNAP5	A24:02	FSADIFFFF		Pat38	LOXL1	B15:17	HTQGLSPDCY
	Pat77	CNTNAP5	A26:01	FSADIFFFF	21	Pat123	MKLN1	C02:02	HSKNCCLYVF
	Pat77	CNTNAP5	C12:03	FSADIFFFF		Pat88	MKLN1	C16:01	HSKNCCLYVF
8	Pat07	CNTNAP5	B08:01	FFFFKTTAL	22	Pat21	OR52N5	A01:01	LSPTLNPIVY
	Pat07	CNTNAP5	C07:02	FFFFKTTAL		Pat49	OR52N5	A01:01	LSPTLNPIVY
	Pat07	CNTNAP5	C07:01	FFFFKTTAL	23	Pat66	TRBV5-1	A01:01	ISGHRSVFWY
	Pat77	CNTNAP5	C01:02	FFFFKTTAL		Pat66	TRBV5-1	B58:01	ISGHRSVFWY
	Pat77	CNTNAP5	C12:03	FFFFKTTAL		Pat174	TRBV5-1	A29:02	ISGHRSVFWY
	Pat07	CNTNAP5	C07:02	IFFFFKTTAL		Pat21	UGT2B28	C07:01	FQYHSLNVI
	Pat77	CNTNAP5	C12:03	IFFFFKTTAL	24	Pat79	UGT2B7	A02:01	FQYHSLNVI
	Pat132	ERCC8	A23:01	CVFQSNFQEF		Pat79	UGT2B7	C02:02	FQYHSLNVI
10	Pat38	ERCC8	C02:02	CVFQSNFQEF	25	Pat88	ZIM3	A03:01	FIYKSDFVK
	Pat38	ERCC8	B15:17	CVFQSNFQEF		Pat38	ZIM3	A03:01	FIYKSDFVK
11	Pat132	ERCC8	A01:01	FQSNFQEFY	26	Pat38	ZIM3	B15:17	KSDFVKHQRI
	Pat38	ERCC8	C02:02	FQSNFQEFY		Pat88	ZIM3	C08:02	KSDFVKHQRI
12	Pat117	FAM5B	A02:01	FQDSALLQL	27	Pat38	ZIM3	B15:17	KAFIYKSDFV
	Pat117	FAM5B	C07:01	FQDSALLQL		Pat88	ZIM3	C16:01	KAFIYKSDFV
	Pat117	FAM5B	C02:02	FQDSALLQL	28	Pat88	ZNF229	A29:02	RVHTGEKLY
	Pat123	FAM5B	C04:01	FQDSALLQL		Pat38	ZNF235	B15:17	RVHTGEKLY
	Pat123	FAM5B	C02:02	FQDSALLQL					

both the cytolytic activity of local immune infiltrates and the neoantigen load (14, 15). Both genes were significantly enriched in the cohort treated with ipilimumab that had clinical benefit compared with the cohort that showed no clinical benefit ($P = 0.042$; Mann-Whitney) (Fig. 3A). Also, the cohort that had long-term survival expressed these cytolytic genes in amounts similar to those of the clinical-benefit group. Expression of CTLA-4 itself and of the programmed cell death 1 ligand 2 (PD-L2), which inhibits peripheral T cell proteins, was also significantly elevated in the cohort that showed clinical benefit compared with the one that showed no clinical benefit ($P = 0.033$ and $P = 0.041$; Mann-Whitney) (Fig. 3B). HLA class I proteins were expressed in similar amounts across clinical response groups ($P > 0.05$ for all; Mann-Whitney) (Fig. 3C).

Together, these findings suggest that expression of immune checkpoint molecules themselves may correlate with immunotherapy response. Overall, our findings imply that both DNA- and RNA-level genomic information may have predictive value for ipilimumab-based therapy. In contrast, recurrent neoantigens (e.g., those occurring in more than one ipilimumab responder) were rare in our cohort—and those that were observed were not necessarily matched by HLA type. These results suggest that the discovery of specific or recurrent neoantigens that mediate response to immunotherapy may require patient cohorts that are much larger in size than those currently available, especially given the importance of HLA restriction in mediating neoantigen recognition (10). Incorporation of patient-matched RNA-level information may help prioritize putative

neoantigens and elucidate possible tumor immune microenvironmental effects, including interferon-related genes and additional immune checkpoint molecules that are relevant in melanoma (28). Additionally, prediction of neoantigens originating from fusion products and class II neoantigens may further inform genomic correlates of response (29–31). In this study, we observed a distinct set of patients who experienced survival for a long time after treatment, despite the early progression of disease. Patterns of initial increases in tumor mass or delayed responses to therapy have been observed after cancer immunotherapy in the past (32). Thus, these observations raise the possibility that neoantigen loads or tumor cytolytic and immune checkpoint expression may play meaningful functional roles in this context,

Fig. 3. Immune microenvironment cytolytic and immune activity correlates with response to ipilimumab. (A) Patients who achieved clinical benefit from immune checkpoint blockade therapy had significantly higher levels of tumor cytolytic activity than those who had minimal or no benefit from ipilimumab ($P = 0.039$). (B) Patients who achieved clinical benefit from ipilimumab therapy had significantly higher levels of expression of immune checkpoint receptors than those who did not (CTLA-4: $P = 0.033$, PD-L2: $P = 0.041$). One point is not shown because of an outlying high CTLA-4 expression value in a nonresponder patient (>50 reads per kilobase per million mapped reads). (C) Response to ipilimumab did not correlate with expression of or mutations in HLA alleles ($P > 0.05$ for all). Asterisks (*) indicate $P < 0.05$.



although these findings require further exploration in larger clinical cohorts. Additional studies of clinical response and resistance to immune checkpoint inhibitors may benefit from integrating exome and transcriptome sequencing data to inform the relative contributions of tumor immunogenicity and host immune infiltration in determining clinical benefit.

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performed sequencing. B.S., C.B., L.Z., A.S., M.H.G.F., S.M.G., J.U., J.C.H. B.W., K.C.K., C.L., P.M., R.G., R.D., and D.S. developed the clinical samples cohort. U.H. performed pathology review on the samples.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S5

Tables S1 to S5

References (35–41)

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ONCOGENE SIGNALING

Identification of an oncogenic RAB protein

Douglas B. Wheeler,^{1,2,3*} Roberto Zoncu,⁴ David E. Root,³ David M. Sabatini,^{3,5,6,7,8†} Charles L. Sawyers^{1,8†}

In a short hairpin RNA screen for genes that affect AKT phosphorylation, we identified the RAB35 small guanosine triphosphatase (GTPase)—a protein previously implicated in endomembrane trafficking—as a regulator of the phosphatidylinositol 3'-OH kinase (PI3K) pathway. Depletion of RAB35 suppresses AKT phosphorylation in response to growth factors, whereas expression of a dominant active GTPase-deficient mutant of RAB35 constitutively activates the PI3K/AKT pathway. RAB35 functions downstream of growth factor receptors and upstream of PDK1 and mTORC2 and copurifies with PI3K in immunoprecipitation assays. Two somatic RAB35 mutations found in human tumors generate alleles that constitutively activate PI3K/AKT signaling, suppress apoptosis, and transform cells in a PI3K-dependent manner. Furthermore, oncogenic RAB35 is sufficient to drive platelet-derived growth factor receptor α to LAMP2-positive endomembranes in the absence of ligand, suggesting that there may be latent oncogenic potential in dysregulated endomembrane trafficking.

The phosphatidylinositol 3'-OH kinase (PI3K)/AKT pathway is a key regulator of cell survival, proliferation, and growth and is commonly activated in human cancers by receptor tyrosine kinase (RTK) signaling

(1, 2). To identify previously unknown regulators of PI3K/AKT signaling, we performed an RNA interference (RNAi) screen using lentiviruses that express short hairpin RNAs (shRNAs) (3) targeting genes coding for all known G proteins

and lipid or protein kinases. Because AKT phosphorylation is a faithful indicator of PI3K activity, we used an immunofluorescent approach to quantitatively measure phosphorylation of AKT at serine 473 (S473) (4) in HeLa cells, which display robust growth factor-induced AKT phosphorylation and lack mutations in core PI3K signaling pathway genes (Fig. 1A). We screened

a collection of 7450 shRNAs in an arrayed format in triplicate to identify kinases or guanosine triphosphatases (GTPases) whose depletion could alter PI3K-dependent AKT phosphorylation.

To identify shRNAs that altered AKT phosphorylation (i.e., “hits”), we translated the phospho-AKT signal in each screened well to a *z* score (5). shRNAs targeting genes that positively regulate AKT phosphorylation (pAKT)—*RICTOR*, *PIK3CA*, *PIK3CB*, *AKT1*, and *AKT3*—all diminished AKT phosphorylation and scored as “pAKT-low” hits (Fig. 1B and tables S1 to S3). Conversely, shRNAs targeting genes whose protein products inhibit AKT signaling—such as *RAPTOR* and *RHEB*—elevated phospho-AKT levels and scored as “pAKT-high” hits. Thus, our screen successfully identified known regulators of PI3K/AKT signaling, as well as a number of genes not previously implicated in regulation of the pathway (table S4). We prioritized hits for genes that had no reported link to PI3K/AKT signaling (table S5) and searched oncogenomic databases to identify those with

somatic mutations reported in human cancers (6, 7). This left us with a list of 29 genes (table S6), 26 of which were pAKT-low hits. Next, we grouped the 26 pAKT-low hits by their annotated functions from the literature and found that the best-represented functional group was the RAB GTPases, which are well known to regulate intracellular protein trafficking (Fig. 1C). This finding suggests that dysregulated protein trafficking by mutant RAB proteins could play a role in oncogenesis.

Of the five RAB proteins that appeared in our list in table S6 (see also Fig. 1C), we pursued RAB35 because, in addition to meeting all of the criteria mentioned previously, it is a well-studied GTPase for which reagents and tools are readily available. RAB35 is a regulator of cytoskeletal organization and trafficking at the recycling endosome (8–11). Endomembrane trafficking by RAB35 is regulated by the DENND1 family of RAB35 guanine nucleotide exchange factors (9, 12–14) and by the TBC1D10 family of RAB35 GTPase activating proteins (15–17). Because RAB35 was

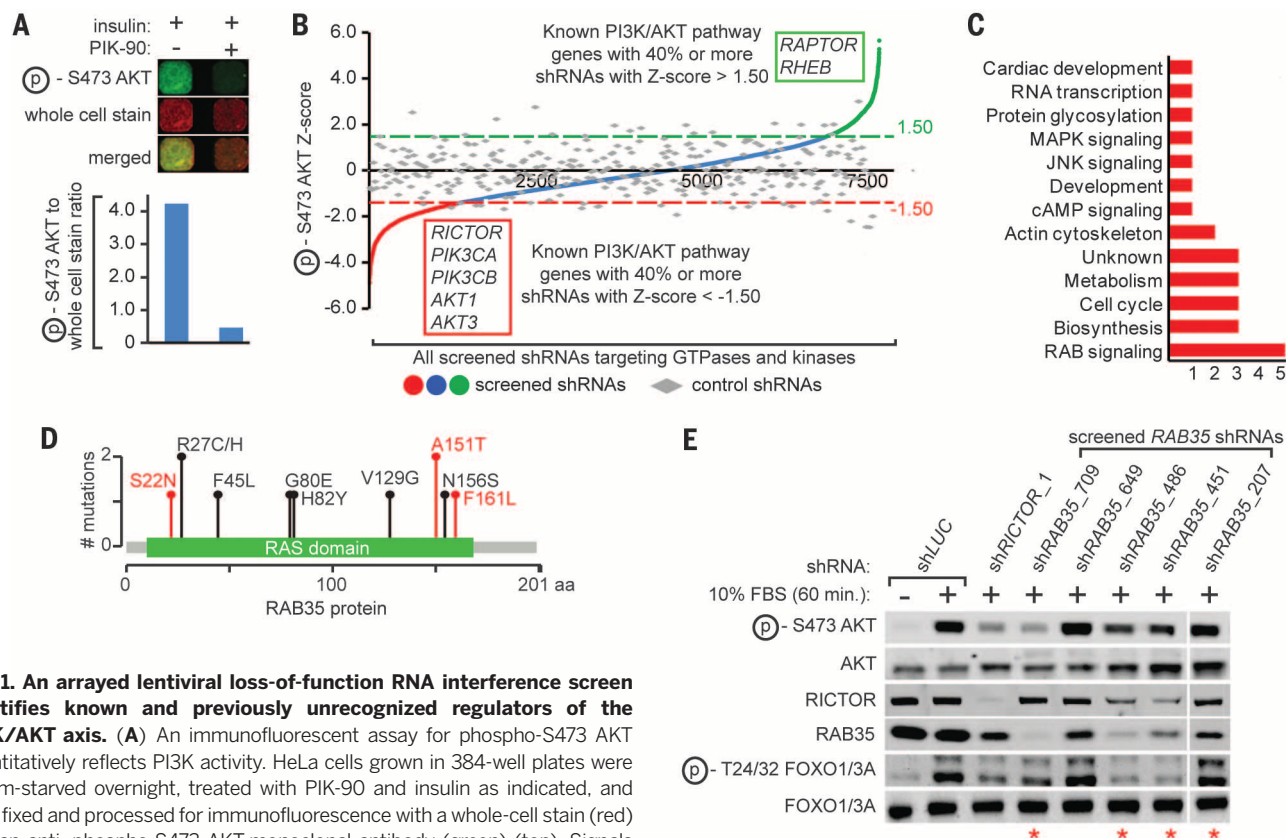


Fig. 1. An arrayed lentiviral loss-of-function RNA interference screen identifies known and previously unrecognized regulators of the PI3K/AKT axis.

(A) An immunofluorescent assay for phospho-S473 AKT quantitatively reflects PI3K activity. HeLa cells grown in 384-well plates were serum-starved overnight, treated with PIK-90 and insulin as indicated, and then fixed and processed for immunofluorescence with a whole-cell stain (red) and an anti-phospho-S473 AKT monoclonal antibody (green) (top). Signals were imaged and quantified with a near-infrared scanner, and the phospho-AKT signal intensity was divided by the whole-cell stain signal intensity to calculate a normalized phospho-AKT value for each well (bottom). (B) Averaged ($n = 3$ replicates) phospho-AKT *z* scores of screened shRNAs. HeLa cells were plated in 384-well plates, transduced with lentiviral reagents expressing control shRNAs or shRNAs targeting human kinases and GTPases, and then processed as in (A). Phospho-AKT *z* scores for each shRNA were calculated, averaged, and arranged as indicated. (C) Functional grouping of the 26 genes whose knockdown lowered AKT phosphorylation, that are not known to regulate PI3K/AKT signaling, and that are mutated in human cancers. Numbers indicate the number of genes in each functional group. MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; cAMP, adenosine 3',5'-monophosphate. (D) Representation of somatic mutations in RAB35 in cancer found in oncogenomic databases. Mutations predicted by the cBioPortal for Cancer Genomics to have a medium effect on protein function are noted in black, whereas mutations predicted to have a high effect are noted in red. Table S7 contains a full list of somatic mutations of RAB35 found in cancers. aa, amino acids. (E) shRNAs targeting *RAB35* deplete *RAB35* protein levels and inhibit AKT phosphorylation. HeLa cells were transduced with lentiviruses expressing the indicated shRNAs, starved overnight and treated with serum as indicated, and lysed; cell lysates were analyzed by immunoblotting. Red asterisks indicate shRNA “hits” from our screen. FBS, fetal bovine serum.

a robust hit in our screen (fig. S1A), has not been implicated as a regulator of PI3K/AKT signaling, and has somatic mutations that have been reported in human cancers mapping to residues conserved in other small GTPases (Fig. 1D and table S7), we prioritized RAB35 for further study.

Next, we confirmed that depletion of RAB35 with the same shRNAs that scored in the original screen suppressed AKT phosphorylation to a similar extent as depletion of the mTORC2 component RICTOR (Fig. 1E). Furthermore, additional shRAB35 hairpins not present in the original screen also suppressed AKT phosphorylation, as well as the AKT substrate FOXO1/3A (fig. S1B). Thus, multiple independent shRNAs targeting RAB35 consistently impair AKT phosphorylation in HeLa cells.

To ensure that the effect of RAB35 knockdown on AKT signaling was not cell-type specific, we depleted RAB35 protein in human embryonic kidney (HEK) 293 E cells and in murine NIH-3T3 cells (Fig. 2, A and B). Knockdowns of RAB35 with two different shRNAs diminished AKT phos-

phorylation at S473 in response to serum stimulation in both human and mouse cells and did so more potently than RICTOR. Furthermore, the PI3K-dependent phosphorylation of FOXO1/3A and the SGK1 substrate NDRG-1 was also decreased in cells depleted of RAB35. We expanded this analysis to seven additional cancer cell lines with different mutational backgrounds (oncogenic *PI3KCA* or *KRAS*, deleted *PTEN*, mutated *NF2* or *TP53*) and generally found reduced AKT phosphorylation after RAB35 depletion, with some modest differences that are likely attributable to distinct hairpins in distinct cellular contexts (fig. S2, A to C). Furthermore, AKT phosphorylation at the PDK1 phosphorylation site T308 was consistently reduced by RAB35 knockdown, suggesting that RAB35 may function upstream of PDK1. Collectively, these data indicate that RAB35 is broadly necessary for the efficient activation of PI3K/AKT signaling in response to serum stimulation.

To explore whether RAB35 is sufficient to activate the PI3K/AKT pathway, we generated cell

lines that stably expressed either wild-type RAB35 (RAB35^{wt}) or the dominant active GTPase-deficient, guanosine triphosphate (GTP)-bound RAB35 Q67→L67 (Q67L) mutant (RAB35^{Q67L}). Cells expressing the wild-type RHEB GTPase (RHEB^{wt}), which regulates mTORC1, served as a control. Stable expression of either RHEB^{wt} or RAB35^{wt} did not alter phosphorylation of AKT or extracellular signal-regulated kinase (ERK) in response to growth factor signaling (Fig. 2C and fig. S3). However, expression of the active RAB35^{Q67L} mutant rendered AKT phosphorylation (at both T308 and S473) constitutively elevated and refractory to growth factor deprivation but did not affect ERK phosphorylation. FOXO1/3A phosphorylation was also consistently elevated in serum-deprived cells expressing RAB35^{Q67L}. Therefore, the expression of GTP-bound RAB35 is sufficient to activate PI3K/AKT signaling in cells, even in the absence of growth factors.

The fact that RAB35^{Q67L} results in constitutive phosphorylation of T308 as well as S473 suggests that RAB35 may function upstream of the

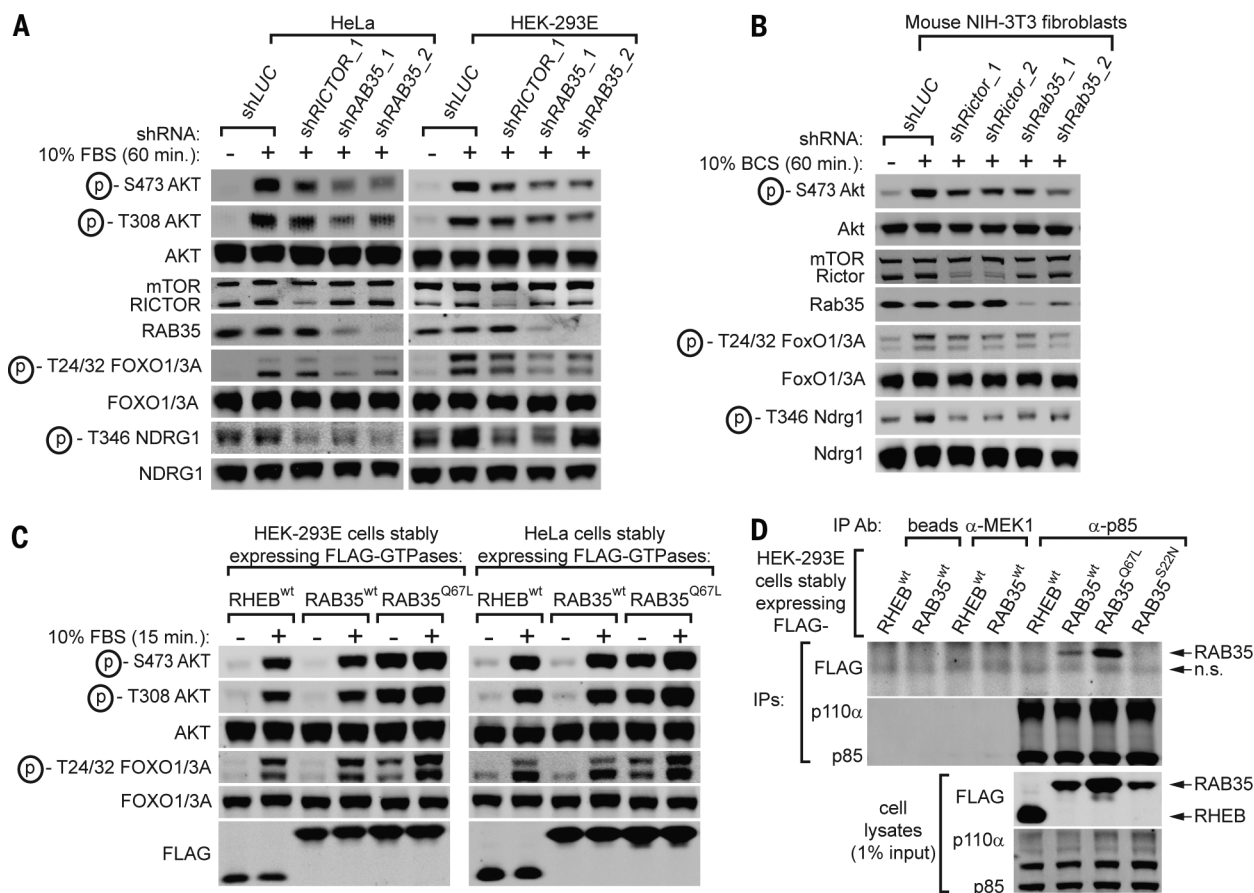


Fig. 2. RAB35 is necessary and sufficient for serum-induced PI3K/AKT signaling and associates with PI3K α . (A and B) Depletion of RAB35 inhibits serum-induced activation of AKT. (A) HeLa and HEK-293E cells were transfected with lentiviral reagents expressing the indicated shRNAs. Cells were serum-starved overnight, treated as indicated with FBS, and lysed; cell lysates were then analyzed by immunoblotting. (B) Murine NIH-3T3 cells were serum-starved then treated as indicated with bovine calf serum (BCS) and analyzed as in (A). (C) GTPase-deficient RAB35^{Q67L} activates PI3K/AKT signaling. HeLa

and HEK-293E cells were stably transduced with lentiviruses expressing FLAG-RHEB^{wt}, -RAB35^{wt}, or -RAB35^{Q67L}. Cells were serum-starved overnight, treated as indicated, and analyzed as in (A). (D) RAB35 associates with PI3K in a nucleotide-dependent manner. Cells stably expressing FLAG-RHEB^{wt}, -RAB35^{wt}, -RAB35^{Q67L}, or -RAB35^{S22N} were lysed, and immunoprecipitations (IPs) were performed using the indicated antibodies. Immunoprecipitates and total cell lysates were then analyzed by immunoblotting with the indicated antibodies. n.s., nonspecific band.

T308 kinase PDK1 (18, 19). However, attribution of RAB35 function to PDK1 versus mTORC2 activation is challenging because the phosphorylation states of T308 and S473 are interdependent (20–22). To address this issue, we took advantage of cells that stably express alleles of murine AKT1 (mAkt1) in which either T308 or S473 were mutated to phosphomimetic aspartate residues (23) (fig. S4, A and B). As expected, mTOR blockade with the TORC1/2 inhibitor torin1 or blockade by RICTOR depletion (resulting in loss of mTORC2 function) suppressed phosphorylation at both sites in mAkt1^{wt}-expressing cells (fig. S4C). In mAkt1^{S473D}-expressing cells, which lack a regulated mTORC2 site, these same interventions either had no effect on T308 phosphorylation (RICTOR depletion) or enhanced T308 phosphorylation (torin 1).

Having confirmed the ability of this system to distinguish direct effects on T308 versus S473, we examined the impact of RAB35 knockdown. Unlike depletion of RICTOR or treatment with

torin 1, depletion of RAB35 decreased T308 phosphorylation in mAkt1^{wt}- and mAkt1^{S473D}-expressing cells. RAB35 depletion also decreased S473 phosphorylation in mAkt1^{wt}- and mAkt1^{T308D}-expressing cells (fig. S4C). These data suggest that RAB35 signals to AKT by functioning upstream of both PDK1 and mTORC2.

We reasoned that if RAB35 acts upstream of both PDK1 and mTORC2, it may be controlling their common regulator, PI3K. Although we could not detect an interaction between endogenous RAB35 and PI3K, we found that stably expressed RAB35, but not RHEB, copurified with PI3K when the kinase was immunoprecipitated by its p85 subunit (Fig. 2D). Interestingly, more FLAG-tagged RAB35 (FLAG-RAB35) copurified with PI3K in immunoprecipitates from cells expressing constitutively GTP-bound RAB35^{Q67L} than from cells expressing RAB35^{wt}. Conversely, we found that the same experiment performed using lysates from cells stably expressing a constitutively guanosine diphosphate-bound allele of

RAB35 (RAB35^{S22N}) did not recover any FLAG-RAB35. Thus, the physical association of RAB35 and PI3K is nucleotide-dependent. We also asked whether RAB35 is necessary for PI3K kinase activity in vitro. As expected, PI3K immunopurified from cells depleted of PI3K α had significantly reduced in vitro kinase activity toward phosphatidylinositol 4,5-bisphosphate (fig. S5A). PI3K purified from cells depleted of RAB35 had a ~50% reduction in PI3K kinase activity in vitro. Further, we found that depletion of RAB35 did not reduce the amount of p110 α recovered in p85 immunoprecipitates, which suggests that RAB35 depletion does not disrupt the p85-p110 α interaction (fig. S5B). Thus, RAB35 copurifies with PI3K in a nucleotide-dependent manner and may be required for optimal PI3K enzymatic function.

Given that RAB35 functions upstream of PI3K, we investigated whether RAB35 acts downstream of any particular growth factor receptor. Depletion of RICTOR, PI3K α , or RAB35 in HeLa cells reduced S473 phosphorylation of AKT in response

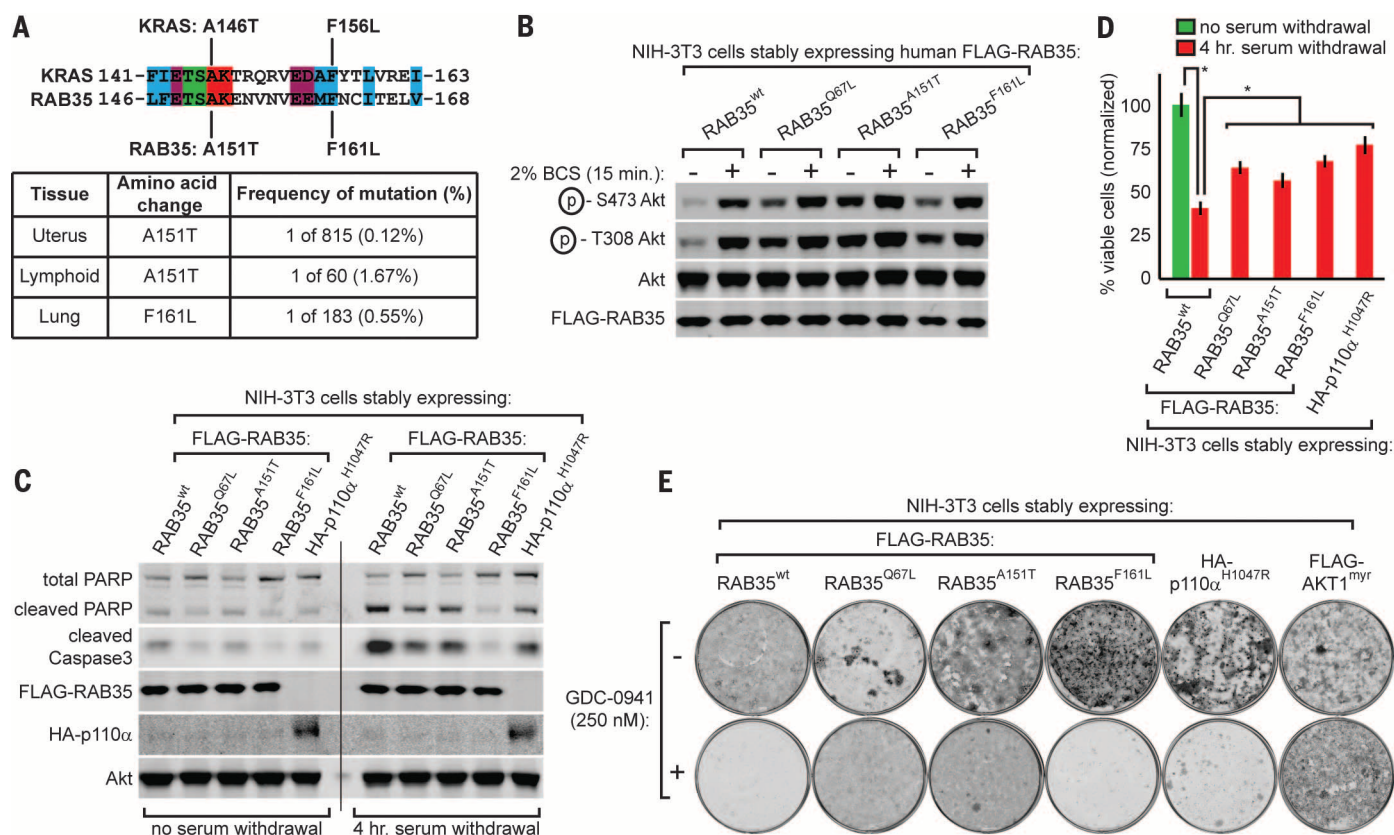


Fig. 3. RAB35 mutants identified in human tumors activate PI3K signaling, suppress apoptosis, and are transforming in vitro. (A) Two mutations in RAB35 that code for amino acid changes A151T and F161L were identified in the MSKCC cBio and COSMIC data sets. Alignment of RAB35 with KRAS was performed using ClustalW2. (B) Mutant RAB35 alleles from human tumors can activate PI3K/AKT signaling. NIH-3T3 mouse fibroblasts stably expressing the indicated RAB35 alleles were serum-starved for 4 hours, left unstimulated or treated with BCS, and lysed; cell lysates were analyzed by immunoblotting for the indicated proteins. (C and D) Expression of RAB35 mutants suppresses apoptosis. (C) NIH-3T3 cells stably expressing the indicated proteins were plated and treated as indicated for 4 hours with or without serum-

containing medium. Cells were then lysed, and the cell lysates were immunoblotted for the indicated proteins. (D) NIH-3T3 cells were treated as in (C) and trypsinized, and the number of viable cells was counted. Cell counts were normalized to the number of viable cells for each cell line from non-serum-starved conditions. Graph bars represent mean normalized cell count; error bars indicate SD ($n = 3$ replicates); and asterisks indicate statistical significance ($P < 0.05$), as determined by a Student's t test. (E) RAB35 mutants can transform cells in vitro in a PI3K-dependent manner. NIH-3T3 cells stably expressing the indicated RAB35 alleles, HA-p110 α ^{H1047R}, or myristoylated FLAG-AKT1^{myr} were cultured for 4 weeks in medium containing 2% BCS with either dimethyl sulfoxide (DMSO) or 250 nM GDC-0941 (a pan-PI3K inhibitor), then fixed, stained with crystal violet, and imaged.

to treatment with either insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), platelet-derived growth factor AA (PDGF-AA), or vascular endothelial growth factor (VEGF) (fig. S6A). RAB35 depletion did not dramatically alter phosphorylation of ERK or tyrosine phosphorylation of receptors for IGF, EGF, PDGF, or VEGF (IGFR, EGFR, PDGFR, or VEGFR, respectively) (fig. S6, B and C). Collectively, these data suggest a model in which RAB35 functions upstream of PI3K and downstream of growth factor RTKs to activate AKT.

One criterion for prioritization of genes on our hit list (table S6) was evidence for mutation in human cancer databases (table S7). For *RAB35*, missense mutations have been reported at two

residues that are conserved in RAS-like GTPases, A151T and F161L. We noticed that the A151T and F161L mutations in *RAB35* were markedly similar to two well-documented *KRAS* mutations (A146T and F156L) that have been previously identified in human tumor samples (Fig. 3A) (24, 25). Although they are not canonical activating mutations, stable expression of either of these two *KRAS* mutants was sufficient to activate ERK signaling and transform NIH-3T3 cells in vitro. We therefore asked whether the two similar mutations in *RAB35* might also be gain-of-function mutations that activate RAB35 signaling.

To test this possibility, we generated NIH-3T3 cells stably expressing either *RAB35*^{wt}, GTPase-deficient *RAB35*^{Q67L}, or the *RAB35*^{A151T} and

RAB35^{F161L} alleles reported in human tumors. As expected from our earlier experiments in human cells, expression of *RAB35*^{wt} did not activate AKT phosphorylation upon serum deprivation, whereas expression of the GTPase-deficient *RAB35*^{Q67L} did (Fig. 3B). Notably, expression of the two naturally occurring *RAB35*^{A151T} and *RAB35*^{F161L} mutants also elevated AKT phosphorylation levels during serum deprivation, indicating that both are gain-of-function alleles sufficient to activate PI3K/AKT signaling.

Because PI3K/AKT signaling inhibits apoptosis, we next examined whether cells expressing mutant *RAB35* alleles are resistant to apoptosis triggered by growth factor withdrawal. Four hours of serum deprivation of NIH-3T3 cells stably

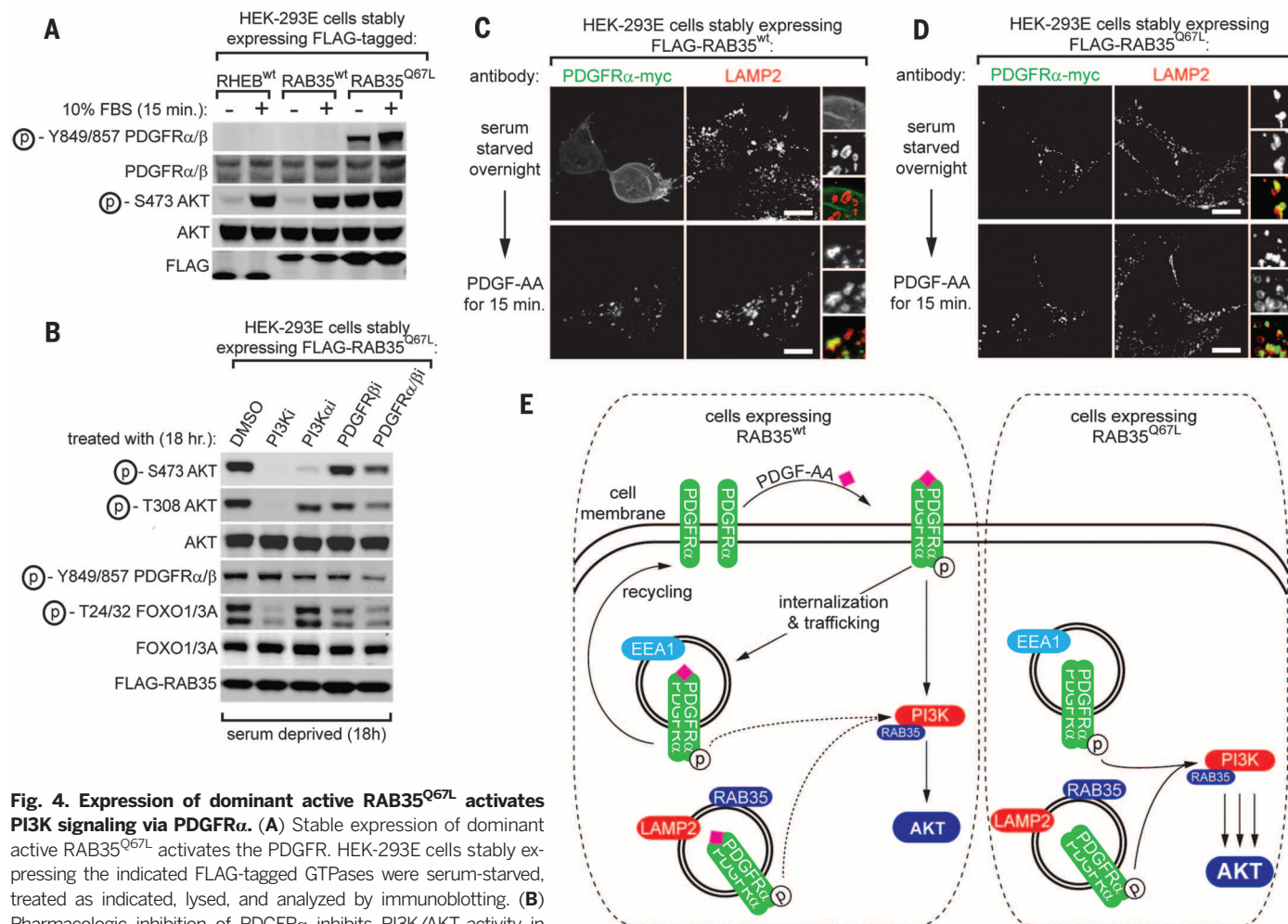


Fig. 4. Expression of dominant active *RAB35*^{Q67L} activates PI3K signaling via PDGFRα. (A) Stable expression of dominant active *RAB35*^{Q67L} activates the PDGFR. HEK-293E cells stably expressing the indicated FLAG-tagged GTPases were serum-starved, treated as indicated, lysed, and analyzed by immunoblotting. (B) Pharmacologic inhibition of PDGFRα inhibits PI3K/AKT activity in cells expressing dominant active *RAB35*^{Q67L}. Cells stably expressing *RAB35*^{Q67L} were treated with DMSO, a pan-PI3K inhibitor (PI3Ki) (GDC-0941, 0.5 μM), a PI3Kα-specific inhibitor (PI3Kai) (BYL719, 1 μM), a PDGFRβ inhibitor (CP-673451, 1 μM), or a PDGFRα/β inhibitor (crenolanib, CP-868596, 1 μM) in serum-free media overnight then lysed and analyzed as in (A). (C) PDGFRα is trafficked to LAMP2-positive endomembranes after stimulation with PDGF. HEK-293E cells stably expressing FLAG-RAB35^{wt} were transfected with cDNA expressing PDGFRα-myc, serum-starved and then treated with PDGF-AA (100 ng/ml) for 15 min, and processed for immunofluorescence with anti-myc and anti-LAMP2 antibodies. (D) Expression of *RAB35*^{Q67L} traffics PDGFRα to LAMP2-positive endomembranes in the absence of PDGF. HEK-293E cells stably expressing FLAG-RAB35^{Q67L} were transfected with cDNA coding for

PDGFRα-myc and then treated and processed for immunofluorescence as in (C). Scale bars in (C) and (D) represent 10 μm. (E) Cartoon depicting the trafficking of PDGFRα to LAMP2-positive endomembranes in cells expressing *RAB35*^{Q67L}. In cells expressing *RAB35*^{wt}, PDGFRα is activated at the cell membrane by PDGF-AA ligand and then internalized to EEA1-positive and LAMP2-positive endomembranes, where liganded PDGFRα drives PI3K/AKT signaling. Dashed lines reflect the fact that endomembrane-based signaling may not be as prominent as plasma membrane signaling in normal states (left). In cells expressing GTPase-deficient, GTP-bound *RAB35*^{Q67L}, unliganded PDGFRα is internalized to EEA1- and LAMP2-positive compartments, where it drives constitutive activation of PI3K/AKT signaling (right).

expressing RAB35^{wt} were sufficient to elevate cleaved levels of the apoptotic markers PARP and caspase 3 (Fig. 3C). In comparison, cells stably expressing one of the three RAB35 mutants or oncogenic p110α^{H1047R} had decreased levels of cleaved PARP and cleaved caspase 3 after serum withdrawal. Further, although cells expressing RAB35^{wt} died in response to serum deprivation, cells expressing mutant alleles of RAB35 or p110α^{H1047R} had significantly improved cell viability when deprived of growth factors (Fig. 3D). Taken together, these data suggest that mutant RAB35 proteins can mitigate cell death in response to growth factor deprivation.

To determine whether the mutant alleles of RAB35 are oncogenic, we examined their activity in a standard NIH-3T3 cell focus-formation assay—an in vitro model of density-independent growth that often correlates with tumorigenicity (26). NIH-3T3 cells expressing oncogenic p110α^{H1047R}, myristoylated AKT1 (AKT1^{myr}), or any of the three mutant alleles of RAB35, but not RAB35^{wt}, formed foci (Fig. 3E and fig. S7). Moreover, we found that when cultured in medium containing 250 nM of the pan-class I PI3K inhibitor GDC-0941 (27), cells expressing RAB35 mutants and p110α^{H1047R} were unable to form foci. Not surprisingly, PI3K blockade did not inhibit the growth of cells transduced with AKT1^{myr}. Thus, the expression of the RAB35 mutants identified in human cancers can transform NIH-3T3 cells in vitro in a PI3K-dependent manner.

Because RAB proteins regulate endomembrane trafficking, we considered whether our evidence implicating RAB35 in AKT activation, downstream of RTKs and upstream of PI3K, is linked to this more established function of RAB proteins. The fact that many RTKs undergo ligand-stimulated internalization and recycling through endosomes, while remaining competent to signal (28–32), suggests that RAB35 may function in this process. In considering this possibility, we noted highly elevated levels of tyrosine phosphorylation of the PDGFRα/β—but not of IGF1R, fibroblast growth factor receptor, VEGFR, or MET—in cells expressing the constitutively active RAB35^{Q67L} allele (Fig. 4A and fig. S8). This increase in PDGFRα/β phosphorylation was markedly reduced by treatment of cells with crenolanib (CP-868596), a PDGFR inhibitor that affects both PDGFRα and PDGFRβ with similar potency (33–35) (Fig. 4B and fig. S9). Notably, crenolanib treatment also diminished phosphorylation of AKT and the downstream substrate FOXO1/3A, suggesting that the RAB35^{Q67L} allele activates AKT through PDGFR. Treatment with a more β-selective PDGFR inhibitor (CP-673451) only moderately affected AKT phosphorylation, implicating PDGFRα as the most likely RAB35^{Q67L} target. The pan-PI3K inhibitor (GDC-0941) and the PI3Kα-specific inhibitor (BYL719) both suppressed AKT phosphorylation but not PDGFRα/β phosphorylation, providing further evidence that PDGFR/RAB35^{Q67L} functions upstream of PI3K.

We postulated that RAB35^{Q67L} might initiate PDGFR-mediated AKT activation by altering PDGFRα localization. To explore this possibility,

we transfected cells that stably expressed either RAB35^{wt} or RAB35^{Q67L} with cDNA encoding for myc-tagged PDGFRα and studied the localization of tagged PDGFRα in the presence or absence of the PDGFRα ligand PDGF-AA (fig. S10). As expected, in cells that expressed RAB35^{wt}, stimulation with PDGF-AA relocalized PDGFRα into punctate intracellular structures. Notably, PDGFRα was constitutively localized to punctate structures in RAB35^{Q67L}-expressing cells, even in the absence of PDGF-AA stimulation. This effect was specific to PDGFRα because internalization of EGFR was not altered by RAB35^{Q67L} expression (fig. S11). By simultaneously visualizing PDGFRα-myc with the lysosomal protein LAMP2 or the early endosome marker EEA1, we next explored whether the punctate localization of PDGFRα in RAB35^{Q67L}-expressing cells was the same as that seen in the normal physiologic context of PDGFRα activation by PDGF-AA. In both PDGF-AA-stimulated RAB35^{wt} cells and unstimulated RAB35^{Q67L}-expressing cells, transfected PDGFRα-myc colocalized with endogenous LAMP2 (Fig. 4, C and D, and fig. S12) and EEA1 (fig. S13). We also found that FLAG-RAB35 is present in endomembranes that contain LAMP2 (fig. S14A) but not in those that contain EEA1 (fig. S14B). This suggests that PI3K activity downstream of RAB35^{Q67L} may originate on LAMP2-positive membranes. Finally, because it has been established that PI3K activity is required for the internalization and trafficking of PDGFRs (36, 37), we asked whether the localization of PDGFRα-myc in cells expressing RAB35^{Q67L} was a result of the increased PI3K signaling in these cells. Although PI3K was required for ligand-induced PDGFRα internalization in cells that express RAB35^{wt} (fig. S15A), treatment of cells expressing RAB35^{Q67L} with a pan-PI3K inhibitor did not diminish the localization of PDGFRα-myc to LAMP2-positive compartments (fig. S15B). PI3K inhibitor treatment resulted in changes in LAMP2 vesicle morphology that may merit further investigation (fig. S15, A and B). Together, these data establish that activated PDGFRα is normally trafficked through LAMP2-positive endomembranes and that constitutively active RAB35 likely drives unliganded PDGFRα to this location, where it is active and can signal to PI3K/AKT (Fig. 4E). Further study is required to determine whether mutant RAB35 diverts newly synthesized PDGFRα from the cell membrane to LAMP2-positive endomembranes or prevents the exit of existing PDGFRα from LAMP2 compartments.

Here we demonstrate a previously unappreciated role for the small GTPase RAB35 in regulating growth factor signaling to PI3K/AKT. We also characterize two RAB35 missense mutations found in human tumors that confer gain of function in standard transformation assays, displaying phenotypes similar to well-characterized oncogenic KRAS alleles. Searches of existing human tumor sequencing databases reveal that RAB35 mutations are rare (table S7) and are therefore unlikely to appear on current lists of human cancer genes that rely solely on statistical methods to distinguish between driver and passenger mutations. Nonetheless, the conserved location of the RAB35

mutations to analogous residues in KRAS and their oncogenic phenotype in functional assays provide strong evidence that these are true driver mutations and are perhaps clinically actionable on the basis of their sensitivity to PI3K inhibition. Considering the broad role of RAB family proteins in endomembrane trafficking, it is intriguing to speculate that the oncogenic RAB35 phenotype reported here may be a consequence of dysregulated membrane trafficking. Our studies showing that the constitutively active RAB35^{Q67L} allele is sufficient to drive PDGFRα to LAMP2-positive endomembranes, in a pattern that mirrors that seen with native PDGF-AA ligand, are consistent with this model. Alternatively, these mutations may be neomorphic, conferring a novel molecular function to RAB35 that results in signaling in a RAS-like manner. The fact that other RAB proteins (RAB1B, RAB39, RABL3, RASEF) emerged as hits in our screen and have rare missense mutations reported in human tumors (table S6) begs a broader examination of this question.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6257/211/suppl/DC1
Materials and Methods

Figs. S1 to S15

Tables S1 to S7

References (38–55)

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VIROLOGY

Dengue subgenomic RNA binds TRIM25 to inhibit interferon expression for epidemiological fitness

Gayathri Manokaran,^{1,2,3} Esteban Finol,^{1,3,4} Chunling Wang,⁵ Jayantha Gunaratne,^{3,6} Justin Bahl,⁷ Eugenia Z. Ong,¹ Hwee Cheng Tan,¹ October M. Sessions,¹ Alex M. Ward,¹ Duane J. Gubler,¹ Eva Harris,⁵ Mariano A. Garcia-Blanco,^{1,8,9} Eng Eong Ooi^{1,3,10,11*}

The global spread of dengue virus (DENV) infections has increased viral genetic diversity, some of which appears associated with greater epidemic potential. The mechanisms governing viral fitness in epidemiological settings, however, remain poorly defined. We identified a determinant of fitness in a foreign dominant (PR-2B) DENV serotype 2 (DENV-2) clade, which emerged during the 1994 epidemic in Puerto Rico and replaced an endemic (PR-1) DENV-2 clade. The PR-2B DENV-2 produced increased levels of subgenomic flavivirus RNA (sfRNA) relative to genomic RNA during replication. PR-2B sfRNA showed sequence-dependent binding to and prevention of tripartite motif 25 (TRIM25) deubiquitylation, which is critical for sustained and amplified retinoic acid–inducible gene 1 (RIG-I)–induced type I interferon expression. Our findings demonstrate a distinctive viral RNA–host protein interaction to evade the innate immune response for increased epidemiological fitness.

Dengue outbreaks emerge when DENVs are introduced into populations with low herd immunity. However, several outbreaks have ensued when new DENV strains emerged and displaced endemic strains, albeit of the same serotype and genotype (1–6), suggesting that genetic variation altered viral fitness in epidemiological settings.

To gain insights into how DENV genome variation affects its epidemiological phenotype, we examined a DENV-2 clade replacement event that coincided with an epidemic of severe dengue in Puerto Rico in 1994 (1, 7, 8). Phylogenetic analysis of the complete coding sequences of DENV-2 isolated from Puerto Rico and neighboring countries showed that three distinct clades within the Asian/American genotype circulated in 1994 (fig. S1), concordant with previous findings (7). Clade PR-1 was prevalent from 1986 to 1995. Clade PR-2 contained viruses in two subclades, both first isolated in 1994: PR-2A persisted at low levels until 1998, whereas PR-2B spread and replaced PR-1 to become the dominant DENV-2 in Puerto Rico from 1995 to 2007. Immune escape is an unlikely explanation for PR-2B emergence, as both PR-1 and PR-2B belong to the Asian/American genotype. Furthermore, prM and E amino acid sequence analysis showed weak bootstrap values (fig. S2), indicating low levels of antigenic differences between the clades. Instead, PR-2B emergence and subsequent replacement of PR-1 viruses could be due to differences in epidemiological fitness.

To identify the genomic variations that segregated these DENVs into different clades and modulated epidemiological fitness, we applied the Shimodaira-Hasegawa (SH) test (9) that compares the topology of region-specific with full-genome trees. This approach identified NS1, NS3, NS5, and the 3' untranslated region (3'UTR) sequence variations as potentially critical in segregating DENVs into PR-1 and PR-2B (figs. S3 to S5).

NS1, NS3, and NS5 are DENV replication complex components that also regulate the innate immune response (10). Less is known about the 3'UTR. During flaviviral replication, the cellular 5'-to-3' exoribonuclease digests uncapped genomes but stalls at pseudoknot RNA structures in the 3'UTR. The resultant 0.3- to 0.5-kb subgenomic flavivirus RNA (sfRNA) regulates pathogenicity in both mammalian and mosquito cells, likely through mechanisms and structural elements within its noncoding RNA that remain to be fully defined (11–15). Recently, we showed that sfRNA could interact with proteins to inhibit translation of interferon-stimulated genes (16). Because two substitutions identified in the PR-2B 3'UTR were located where pseudoknots could form (fig. S6) (17–19), we tested for a possible role for sfRNA in modulating fitness in our viruses.

To measure viral fitness in vitro, we performed DENV infection assays in human hepatoma (HuH-7) cells. We expected PR-2B DENVs to replicate more efficiently than PR-1 viruses. Paradoxically, however, more viral progeny (Fig. 1A) and genomic RNA (gRNA) (Fig. 1B) were produced by PR-1 than PR-2B viruses at 24 hours postinfection (hpi). The difference in sfRNA levels between PR-1 and PR-2B viruses was smaller, although statistically significant (Fig. 1C), resulting in higher sfRNA:gRNA ratios in PR-2B as compared to PR-1 viruses (Fig. 1D). While the absolute values of the sfRNA:gRNA ratios should be interpreted with caution, our data clearly show that these ratios are markedly higher for the epidemic PR-2B viruses.

Increased production of sfRNA relative to gRNA in an epidemic clade of viruses is not specific to the PR-2B viruses. In Nicaragua, NI-2B DENV-2 emerged abruptly in 2005, displaced the endemic NI-1 DENV-2, and caused increased rates of severe disease across several epidemic seasons (5). SH test on these Asian/American genotype DENV-2 isolates similarly identified variations in NS1, NS5,

¹Program in Emerging Infectious Diseases, Duke–National University of Singapore Graduate Medical School, Singapore. ²Defence Medical and Environmental Research Institute, DSO National Laboratories, Singapore. ³Yong Loo Lin School of Medicine, National University of Singapore, Singapore. ⁴Swiss Tropical and Public Health Institute, Universität Basel, Switzerland. ⁵Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, CA, USA. ⁶Institute of Molecular and Cell Biology, Singapore. ⁷Center for Infectious Diseases, The University of Texas School of Public Health, Houston, TX, USA. ⁸Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX, USA. ⁹Department of Molecular Genetics and Microbiology and Center for RNA Biology, Duke University, Durham, NC, USA. ¹⁰Saw Swee Hock School of Public Health, National University of Singapore, Singapore. ¹¹Singapore–Massachusetts Institute of Technology Alliance in Research and Technology Infectious Disease Interdisciplinary Research Group, Singapore.

*Corresponding author. E-mail: engeong.ooi@duke-nus.edu.sg

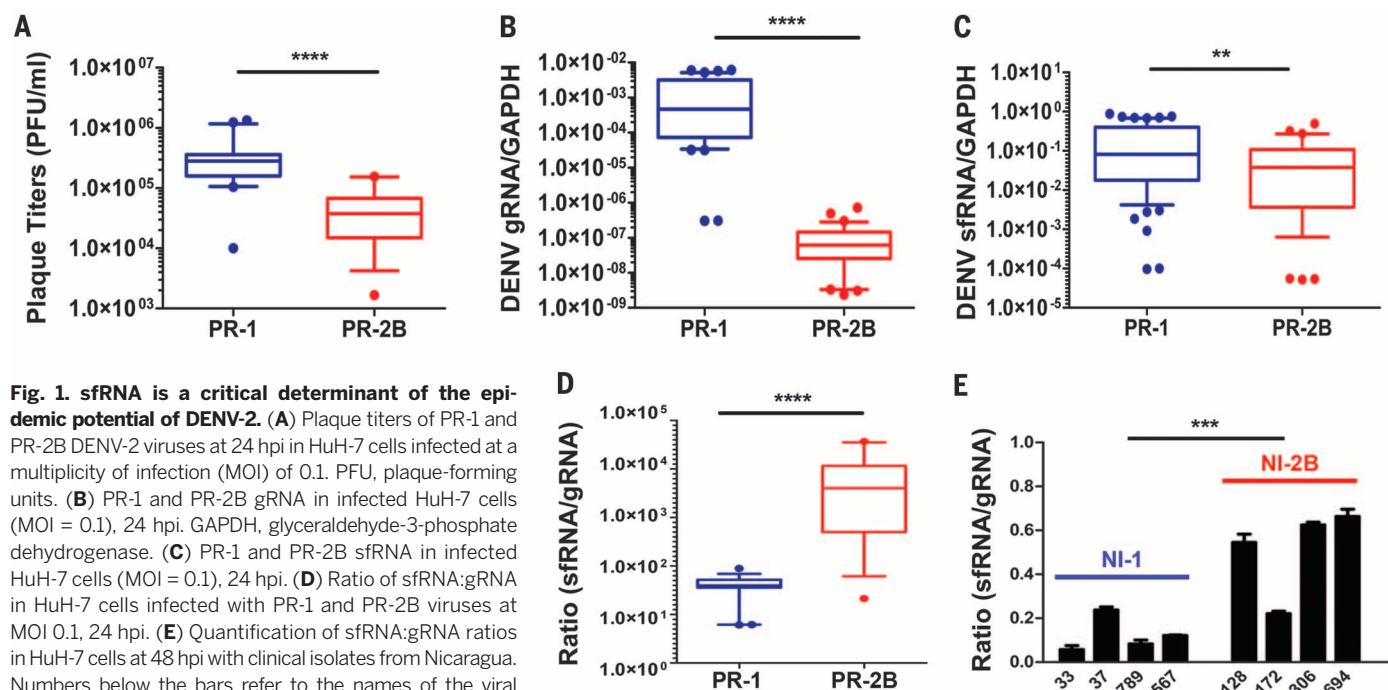


Fig. 1. sfRNA is a critical determinant of the epidemic potential of DENV-2. (A) Plaque titers of PR-1 and PR-2B DENV-2 viruses at 24 hpi in HuH-7 cells infected at a multiplicity of infection (MOI) of 0.1. PFU, plaque-forming units. (B) PR-1 and PR-2B gRNA in infected HuH-7 cells (MOI = 0.1), 24 hpi. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. (C) PR-1 and PR-2B sfRNA in infected HuH-7 cells (MOI = 0.1), 24 hpi. (D) Ratio of sfRNA:gRNA in HuH-7 cells infected with PR-1 and PR-2B viruses at MOI 0.1, 24 hpi. (E) Quantification of sfRNA:gRNA ratios in HuH-7 cells at 48 hpi with clinical isolates from Nicaragua. Numbers below the bars refer to the names of the viral isolates. Data are expressed as mean $\pm$ SD from three independent experiments. ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$, as determined by *t* test.

and 3'UTR as important in segregating these viruses into NI-1 and NI-2B clades (fig. S7 and S8). In this case, two compensatory 3'UTR substitutions in NI-2B may produce energetically more stable secondary RNA structures (fig. S9). Further analysis indicated three- to fourfold higher sfRNA:gRNA ratios upon NI-2B as compared to NI-1 DENV-2 infection (Fig. 1E). Collectively, these data suggest that increased sfRNA production and possibly the sequence or structure of sfRNA may contribute to DENV-2 fitness in distinct epidemiological settings.

To understand how sfRNA production and/or sequence could lead to greater epidemiological fitness, we compared the replication kinetics of selected PR-1 and PR-2B viruses and their effect on interferon expression. Although significant differences in plaque titers and gRNA levels exist at 24 hpi, PR-2B viruses grew at a faster rate than PR-1 such that no difference in either parameter was observed at 96 hpi (Fig. 2, A and B). In addition, interferon-beta (IFN- β) expression was consistently lower in PR-2B- than in PR-1-infected cells, despite diminished differences in DENV gRNA levels at later time points (Fig. 2, C to F). This suggests that higher sfRNA:gRNA ratios produced during PR-2B infection suppressed IFN- β induction. Indeed, silencing interferon regulatory factor-3 (IRF-3), a transcription factor for IFN- β , increased PR-1 but not PR-2B replication (Fig. 2G).

The trends observed in infection of HuH-7 cells were recapitulated in primary monocytes, which are target cells for DENV in humans. Antibody-enhanced infection, which is associated with greater risk of severe dengue, produced less gRNA, greater sfRNA:gRNA ratios, and reduced IFN- β expression at 24 hpi when PR-2B instead of PR-1 viruses were used (Fig. 2, H to J). Likewise, at 72 hpi, differences between the plaque titers of PR-1 and

PR-2B isolates were reduced or even reversed (Fig. 2K).

Given the observed association between increased sfRNA:gRNA ratios and reduced IFN- β expression, we next examined if the three nucleotide substitutions in the 3'UTR could functionally account for the increased sfRNA:gRNA ratios. To eliminate any contributions from NS1, NS3, and NS5, we performed mutagenesis studies on a standardized genomic backbone using a previously characterized DENV-2 replicon reporter, pDENrep-FH (20). At 72 hours after electroporation, replicons with PR-2B residues produced less gRNA but higher sfRNA:gRNA ratios compared to that bearing PR-1 residues (Fig. 3A). Likewise, luciferase signals were lower in replicons with PR-2B instead of PR-1 residues (Fig. 3B), indicating that substitutions in these three nucleotide positions can account for the observed differences in PR-1 and PR-2B sfRNA:gRNA ratios.

To investigate whether sfRNA plays a functional role in modulating IFN- β expression, we co-electroporated in vitro-transcribed sfRNA from PR-1 or PR-2B or a size-matched RNA control with pDENrep-FH into HuH-7 cells. As compared to nuclear factor κ B (NF- κ B) expression, where no difference was seen, IFN- β expression was significantly reduced with PR-2B but not PR-1 sfRNA, at 96 hours after electroporation (Fig. 3C). Correspondingly, pDENrep-FH replication was significantly increased when co-electroporated with PR-2B sfRNA compared to either PR-1 sfRNA or control RNA (Fig. 3D). Furthermore, sfRNA but not size-matched control RNA inhibited polyinosinic-polycytidylic acid (polyIC)-induced IFN- β expression, with a greater difference observed for PR-2B than PR-1 sfRNA (fig. S10 and Fig. 3E). Collectively, these data indicate that both the

amount and sequence of sfRNA are important in attenuating type I interferon expression for increased DENV replication.

The sequence specificity observed suggests that IFN- β suppression is likely to be due to specific sfRNA-protein interactions (27). Using stable isotope labeling by amino acids in cell culture coupled with quantitative mass spectrometry (SILAC-qMS), we detected 198 proteins that were enriched (at least 1.5-fold change) with DENV 3'UTR compared to control RNA (table S1 and fig. S11, A and B) (22). Notably, tripartite motif containing 25 (TRIM25) and mitochondrial antiviral signaling (MAVS) proteins (fig. S11C), which are known retinoic acid-inducible gene 1 (RIG-I) signaling intermediates for type I interferon expression (23–25), were significantly enriched with PR-2B compared to PR-1 3'UTR.

To validate our SILAC-qMS findings, we immunoprecipitated TRIM25 and MAVS separately from DENV-2-infected cells and quantified the protein-bound sfRNA and gRNA. Significantly higher sfRNA than gRNA levels were detected with TRIM25 immunoprecipitation as compared to isotype immunoglobulin G (IgG) control, with the difference being more pronounced when PR-2B DENV was used (Fig. 4A). Conversely, neither sfRNA nor gRNA was enriched with MAVS immunoprecipitation (fig. S12). In addition, incubating PR-2B sfRNA with TRIM25 yielded more RNA-protein complex than PR-1 sfRNA as measured on an electrophoretic mobility shift assay (EMSA) (Fig. 4B), indicating sequence-specific differences in TRIM25 binding.

Besides being an RNA-binding protein (26), TRIM25 is an E3 ligase that, upon deubiquitylation by ubiquitin-specific peptidase 15 (USP15) (27), polyubiquitylates RIG-I for sustained and

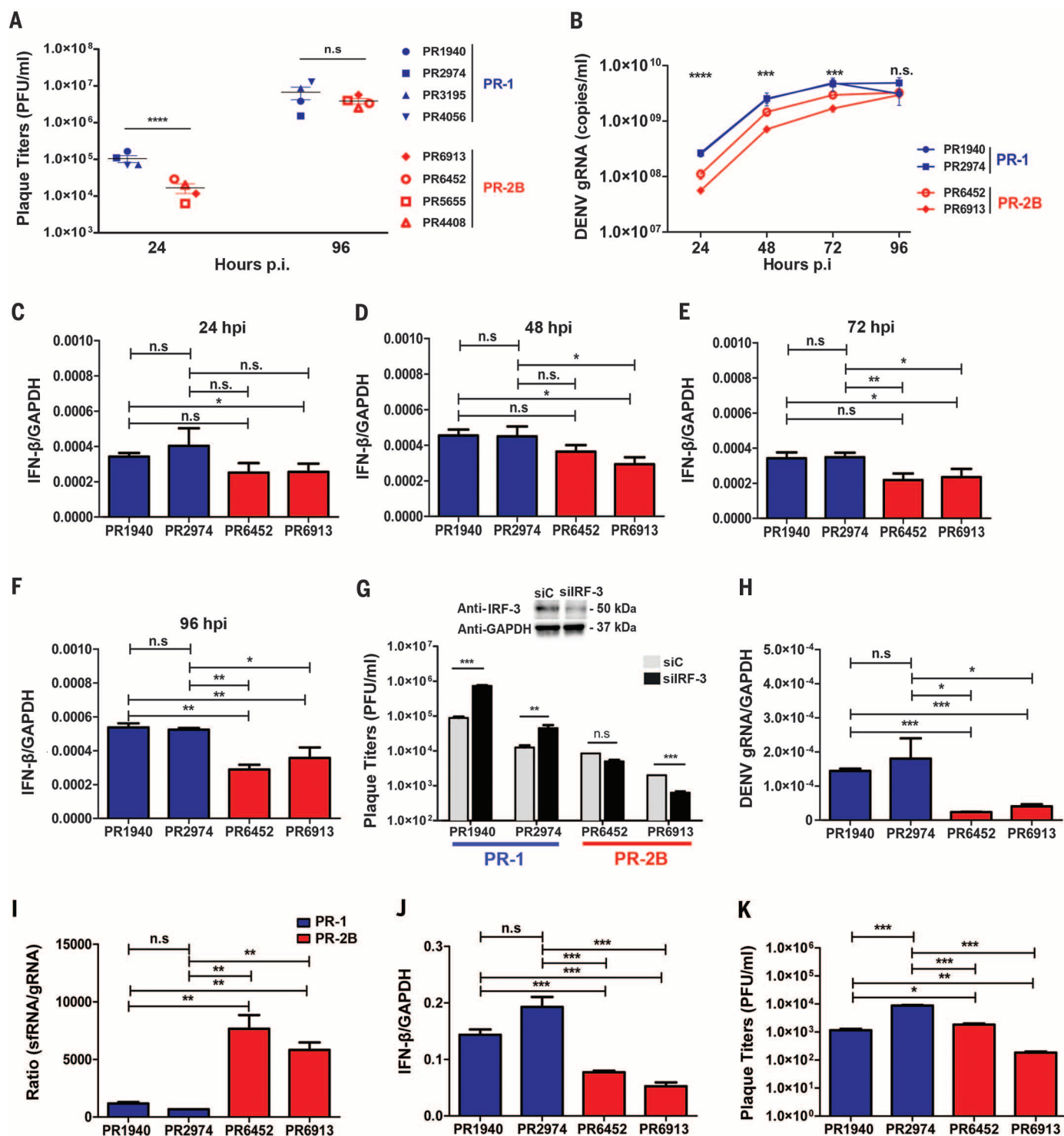


Fig. 2. PR-2B DENV-2 replication is associated with reduced expression of IFN-β relative to PR-1 DENV-2. (A) Plaque titers after infection of HuH-7 cells with selected viruses from each PR clade at 24 and 96 hpi. (B) DENV-2 gRNA levels in infected HuH-7 cells after infection (MOI = 0.5) with selected viruses from each clade at various time points postinfection. (C to F) Expression levels of IFN-β in infected HuH-7 cells at 24 hpi (C), 48 hpi (D), 72 hpi (E), and 96 hpi (F). PR-1: blue bars; PR-2B: red bars. (G) Plaque titers of supernatant quantified at 24 hpi with representative viruses from each clade, following knockdown of IRF-3 in HuH-7 cells by small interfering RNA (siRNA).

siC: control RNA; siIRF-3: siRNA against IRF3. (H) Quantification of DENV-2 gRNA in infected primary monocytes at 24 hpi. Infection was performed with selected PR-1 and PR-2B DENV-2 viruses opsonized with enhancing concentrations of humanized 3H5 monoclonal antibody against DENV-2 E protein. (I) Quantification of sfRNA:gRNA ratios in primary monocytes, 24 hpi. (J) IFN-β expression in primary monocytes at 24 hpi. (K) Plaque titers from supernatant of infected primary monocytes at 72 hpi. Data are expressed as mean ± SD from three independent experiments. n.s.: not significant; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$, as determined by t test.

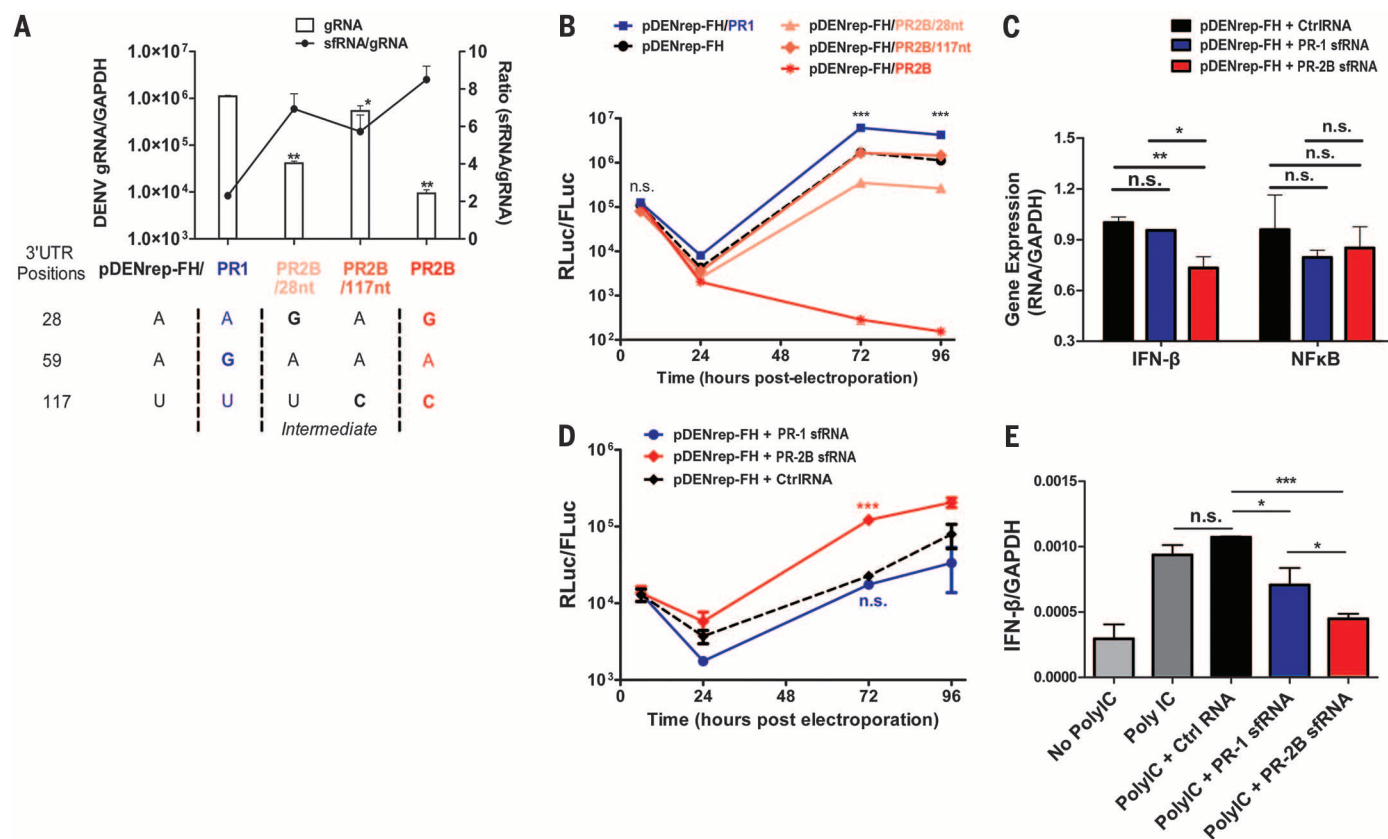


Fig. 3. sfRNA attenuates type I interferon antiviral response, resulting in increased viral replication. (A) Quantification of gRNA levels and sfRNA: gRNA ratios in HuH-7 cells, 72 hours after electroporation with replicons bearing nucleotide residues representative of PR-1 or PR-2B DENV-2. Bars show the gRNA levels (left axis), and lines show sfRNA:gRNA ratios (right axis). (B) Luciferase reporter activity for the original replicon (pDENrep-FH) and mutated replicons at various time points after electroporation. Determination of firefly luciferase (FLuc) levels served as internal normalization controls. (C) Quantification of IFN-β and NF-κB expression using quantitative

reverse transcription–polymerase chain reaction (QRT-PCR) at 96 hours after electroporation and (D) luciferase reporter activity following co-electroporation of pDENrep-FH with control RNA or sfRNA from PR1 or PR2B. FLuc levels served as internal normalization controls. (E) Quantification of IFN-β expression in HuH-7 cells transfected with polyI:C only, polyI:C and size-matched control RNA, or sfRNA from PR-1 or PR-2B, by QRT-PCR at 24 hours after transfection. Data are expressed as mean ± SD from three independent experiments. n.s.: not significant; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, as determined by t test.

amplified signal transduction (24). To examine if sfRNA interferes with these processes, we immunoprecipitated TRIM25 from DENV-2-infected cell lysates and probed for ubiquitin. Results showed a band identical in size to TRIM25 in infected cell lysates (Fig. 4C), although the intensity of the ubiquitylated TRIM25 band is stronger with PR-2B compared to PR-1 DENV (Fig. 4D). Furthermore, RIG-I immunoprecipitation from sfRNA-transfected cell lysates followed by TRIM25 immunoblotting showed that sfRNA did not prevent TRIM25 from binding RIG-I (Fig. 4E). Instead, higher levels of ubiquitylated TRIM25 were coimmunoprecipitated with RIG-I upon transfection of PR-2B relative to PR-1 sfRNA (Fig. 4E). Consistently, silencing TRIM25 did not augment PR-2B DENV-2 replication, whereas PR-1 DENV-2 replication was significantly increased (Fig. 4F). These findings collectively indicate that PR-2B sfRNA binds TRIM25 more efficiently to interfere with its deubiquitylation, thus preventing amplified and sustained RIG-I signaling for type I IFN induction.

Our report provides a mechanistic explanation for increased DENV fitness in an epidemiological

setting. It adds to the general theme by which *Flaviviruses* use their abundant noncoding RNA to bind and inactivate RNA-binding proteins critical for innate immunity (21). TRIM25 is also targeted by influenza virus to inhibit RIG-I signaling, although in this instance, inhibition is mediated by NS1 protein binding to TRIM25 (23). Reducing sustained and amplified IFN expression could thus be important to many viruses, as IFN renders uninfected cells resistant to viral infection.

Based on our findings, we propose a model to explain the 1994 dengue outbreak in Puerto Rico (fig. S13). The high sfRNA:gRNA ratios produced by PR-2B viruses during early phases of the infection constitute a “one-two punch” against host response; greater levels of sfRNA inhibit TRIM25 in a sequence-dependent manner, whereas reduced gRNA results in lower stimulation of RIG-I/melanoma differentiation-associated protein 5 (MDA5) RNA sensors. Reduced IFN responses in the early stages of infection would ensure availability of susceptible cells for viral spread in a human host to reach viremia levels sufficient for further mosquito-borne transmission.

In conclusion, our study provides unique molecular insights into the epidemiological fitness of DENV. It also suggests that by combining epidemiologic studies with molecular investigations, viral phylogenetic information can be informative not only with respect to virus evolution but also as a predictor of its epidemic potential.

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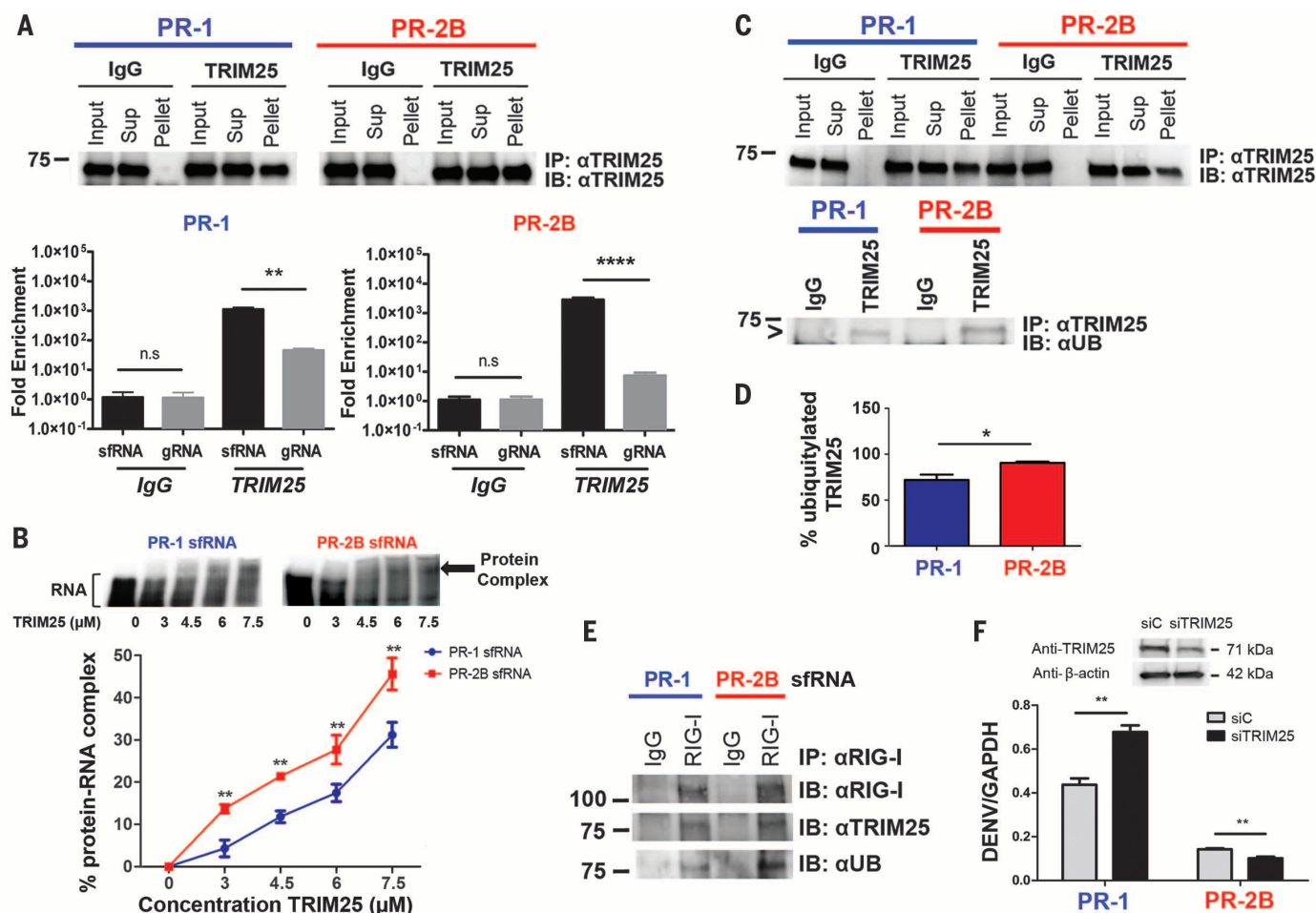


Fig. 4. sfRNA binds TRIM25 to inhibit TRIM25 deubiquitylation. (A) RNA-immunoprecipitation (RIP) was performed on HuH-7 cells at 24 hpi with one virus from each clade (MOI = 5). Infected cells were subjected to TRIM25 or IgG control pull-down followed by immunoblotting with TRIM25 to confirm efficacy of pull-down. Input: cell lysate before RIP; Sup: supernatant after incubation of antibody-coated beads with cell lysate; Pellet: final product after washing. Bar graphs show fold enrichment of sfRNA and gRNA upon pull-down with TRIM25, as measured by QRT-PCR. (B) EMSA of synthetic PR-1 and PR-2B sfRNA (30 nM) incubated with increasing concentrations of TRIM25. Arrow indicates the position of the sfRNA-protein (ribonucleoprotein) complex. Amount of ribonucleoprotein complex was quantified and expressed as a percentage of total RNA signal. (C) Lysates from HuH-7 cells infected with one

virus from each clade (MOI = 5) were subjected to TRIM25 or IgG control pull-down followed by immunoblotting with TRIM25 and ubiquitin (UB). Arrow indicates ubiquitylated bands. (D) Bar chart depicts percentage of ubiquitylated TRIM25 over total TRIM25. (E) After transfection with PR-1 or PR-2B sfRNA, HuH-7 cell lysates were subjected to RIG-I pull-down followed by immunoblotting with RIG-I, TRIM25, and UB. (F) PR-1 and PR-2B DENV-2 replication at 24 hpi in TRIM25-silenced HuH-7 cells infected with one virus from each clade. siC: control RNA; siTRIM25: siRNA against TRIM25. Differences in gel electrophoresis conditions could have led to slight variations in mobility of pre-stained protein markers. Data are expressed as mean $\pm$ SD from three independent experiments. n.s.: not significant; * $P \leq 0.05$, ** $P \leq 0.01$, **** $P \leq 0.0001$, as determined by *t* test.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6257/217/suppl/DC1
Materials and Methods
Figs. S1 to S13
Table S1
References (28–40)

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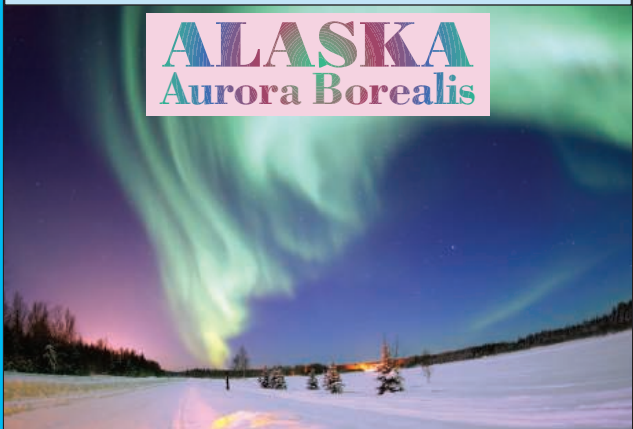
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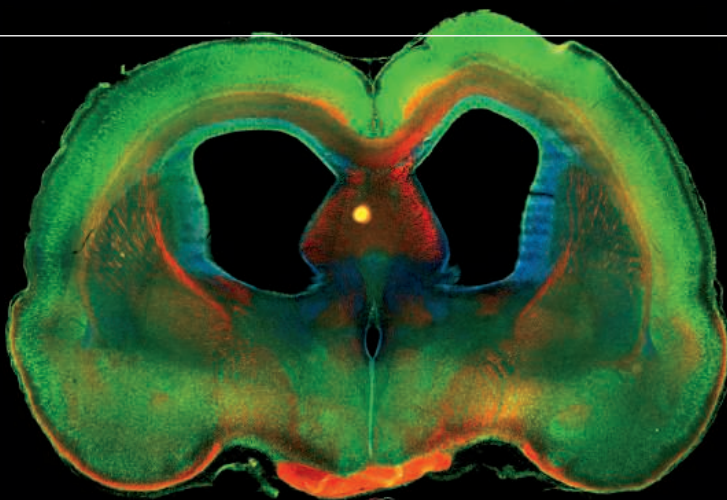


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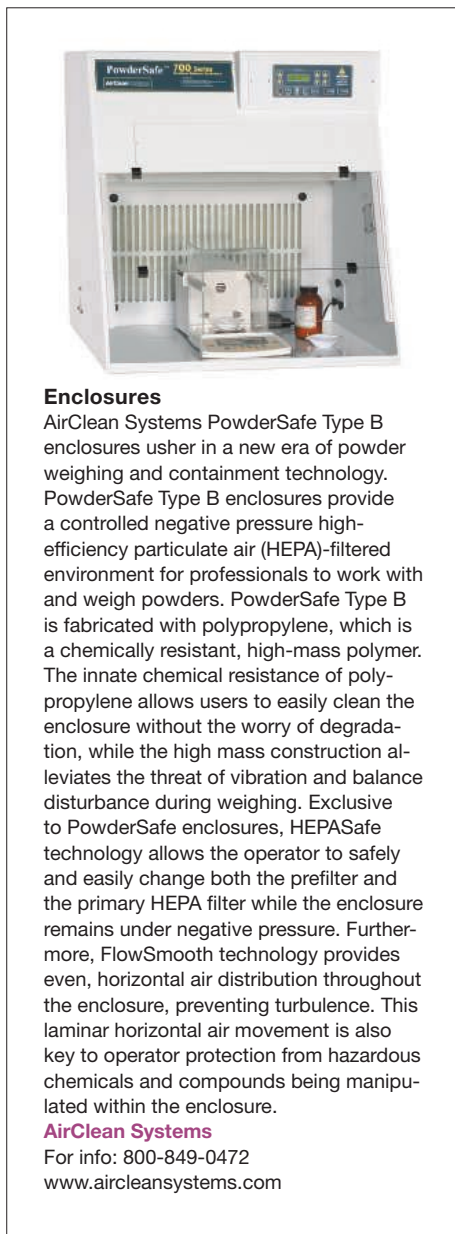
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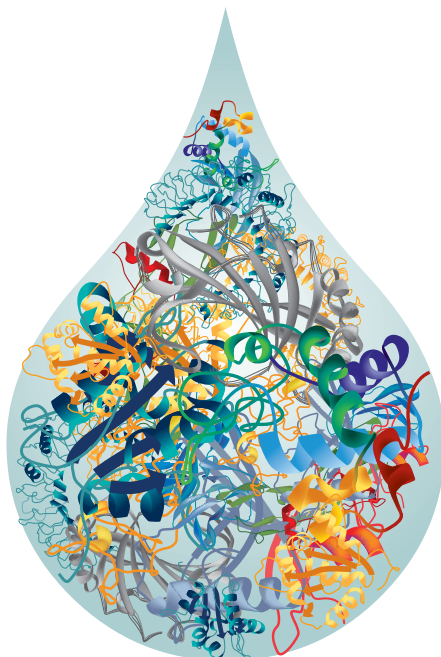
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Industry experience as a platform for academic careers

The contemplative question of whether to pursue a vocation in academia or industry has changed as career paths become more fluid and lines between sectors begin to blur. Scientists and engineers who have spent time in private and public companies are finding professional opportunities open to them in academia. Their value in higher education is varied and appreciated, as they can provide unique perspective and skills, access to new networks, and knowledge concerning how to craft win-win partnerships between universities and companies. For researchers ready to leave the halls of industry, the Ivory Tower can be an accessible and welcoming career avenue. **By Alaina G. Levine**

Industry or academia—which to choose for a career? Although the answer to this query might have only been one or the other in the past, today there are many academics who join a university after finding triumph in industry. One such professional avenue is the classic tenure-track position, which **Kentaro Toyama** pursued. A Ph.D. computer scientist, Toyama spent 12 years at Microsoft Research where he conducted tasks very similar to those found at a university, such as publishing and teaching. “More than anything, I was doing research in the same way someone in academia would have been,” he says. His scholarly contributions were known throughout the field and led directly to his appointment in 2015 as the W. K. Kellogg Associate Professor of Community Information at the University of Michigan School of Information.

Other scientists have found their way in (or back) to academia via administrative positions. **Gordon Smith**, professor and head of the Department of Grain Science and Industry and director of the International Grains

Program Institute at Kansas State University (K-State), believed he “could add value in academia after a successful industry career,” he says. While studying synthetic organic chemistry for his Master’s, he met a food scientist who told him about how cherished chemists are in the food industry, where “you can work on products that people can feel, see, and touch,” he recounts. Smith switched his Ph.D. to food science and launched his career with Sara Lee, where he remained for 14 years, rising to the rank of senior director of R&D. He then joined ConAgra Foods as a vice president of R&D. Among his responsibilities were industry-university relations, for which he liaised with academia for student recruitment, continuing education for employees, and research partnerships. When his department was shuttered by the company, he found out about the job at K-State through networking. “It’s not like it was a move I hadn’t considered,” he says. “I was always interested in academia, and I had met all my industry goals.”

Another route to serving in academia is as a “professor of practice.” **Jay Goldberg** spent 13 years in industry as a biomedical engineer and engineering manager before deciding to pursue a Doctorate in biomaterials. While writing his thesis, he joined a start-up as director of technology and quality assurance, but within a year, Marquette University hired him to lead a new professional Master’s degree program. Today he is the director of Marquette’s Healthcare Technologies Management program in the Department of Biomedical Engineering and also serves as a clinical professor. “Many schools are hiring professors of practice who focus on preparing students for the ‘real world’ of engineering,” says Goldberg. “These faculty are typically not on the tenure track and focus on teaching, not on research. Having significant industry experience as an engineer allows these faculty to fill a niche in their college that might have been ignored due to the lack of faculty with such experience.”

There are also opportunities to join a university later in life. **Hans Schmitthenner** worked in industrial research for 27 years for AstraZeneca, Kodak, and Carestream Molecular Imaging. When his division disbanded in 2010, the Ph.D. chemist reached out to a former colleague, the chair of the School of Chemistry and Materials Science at Rochester Institute of Technology (RIT), and secured a position as adjunct professor. His knowledge of molecular imaging—a new field that he had learned in his corporate experience—proved to be extremely valuable to his new institution. He was given an opportunity to start a research lab and teach students novel techniques in this discipline. Ultimately two units, the Chester F. Carlson Center for Imaging Science and the School of Chemistry and Materials Science, created a new job just for him, to leverage his knowledge and aid the expansion of an undergraduate research program. He is now an associate research professor at RIT.

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Negotiation and a creative mind can enable an industrial scientist to orchestrate even the most unusual employment arrangements, as physicist **Michael Steel** learned. He was able to craft a deal with his employer (an optics firm) and the University of Sydney to allow him to work three days a week for the firm and two days a week at the university. He was given the title of “honorary associate” and provided space at the university to conduct research and mentor graduate students and postdocs. He did this for four years until he was approached by the photonics group at Macquarie University in Sydney, which was looking to expand, where he joined as an associate professor.

Notably, in certain regions around the world, industry experience is not only highly coveted but is a requirement for academic jobs. In Germany, for example, universities of applied sciences (*Fachhochschule* or *Hochschule für angewandte Wissenschaften* in German) require professors to have at least three years of employment in industry, notes **Klemens Gintner**, a professor of mechanical engineering and mechatronics at the Karlsruhe University of Applied Sciences. Similar obligations exist in the higher education systems of other European nations.



Melody Baglione

Providing unique value

Since most science faculty have spent the majority of their careers in academia, those who have worked outside the Ivory Tower find they have a variety of experiences to offer their new employers. In particular, “pharma and biotech executives bring experience in managing multidisciplinary projects and individuals with

different skill sets,” says **Jared Kaleck**, senior director for discovery at search firm Klein Hersh International. “My experience in organizational, budgeting, and personnel management has made me a more effective leader of a research group,” echoes **Jacquelyn S. Fetrow**, provost and vice president of academic affairs at the University of Richmond. She started her career as a biology professor at State University of New York (SUNY) Albany before leaving to co-launch a biotech software company for which she served as chief scientific officer. Smith knew the importance of collaborating with professionals beyond the world of science, and there he connected with individuals in marketing and external relations to accelerate his department’s growth efforts.

Industry experience helps educational programs expand in imaginative ways. “Industry contacts can be used to develop collaborations between the college and companies,

and recruit sponsors for student design projects and guest speakers for courses,” says Goldberg. Moreover, “sharing industry experience allows one to demonstrate the relevance of topics covered in courses.”

Melody Baglione, associate professor of mechanical engineering at The Cooper Union for the Advancement of Science and Art, agrees. “What engineering education is lacking is a connection between knowledge and real-world problems,” she notes. In her classes she incorporates case studies that she worked on as an engineer for Chrysler. The result is an innovative learning experience in which she “creates project-based learning opportunities that simulate the industry environment.”

Faculty with company expertise also provide much-needed career guidance. “Engineers...who have been involved in the design and development of new technologies and products have a lot to share with engineering students contemplating a similar career path,” says Goldberg. Furthermore, professors can tap their networks to aid students in finding employment.

Those industry contacts are indispensable for other purposes too, including research partnerships and financial investment. “Academic institutions are attracted to professionals who can bring funding with them to the university,” says Kaleck. When **Zhiqiang An** joined The University of Texas Health Science Center at Houston (UTHealth) Medical School as professor of molecular medicine, Robert A. Welch Distinguished University Chair in Chemistry, and director of the Texas Therapeutics Institute, he had spent 11 years at Merck as a director and had authored two books. And yet, because of his lack of an established research program, “I knew I wasn’t able to compete for typical research funding, such as from the National Institutes of Health [NIH],” says Ang. “So instead of spending all my time applying for NIH grants, I reached out to industry. I knew big pharma was looking for academics for collaborative research.” He netted \$5–6 million in his first five years thanks to his network.

Changing environments

With any new environment comes a taste of culture shock and new challenges. In industry, “engineers work on teams with a sense of urgency to bring new products to market,” observes Baglione, while in academia, professors are “expected to simultaneously teach, build a funded research program, publish, and prepare students for their futures.” Moreover, Toyama acknowledges how different decision making is. “At a university, it’s very difficult for decisions to be made without ten faculty agreeing,” he says. “But in a company, you don’t need the approval of everyone.”

Furthermore, “in a company, it can take anywhere from 3–5 years and sometimes as little as a year or less to move a product out,” says Steel. “But in academia, outcomes may come in 10–15 years. The impact at the university is important, but unless you’re in an area close to commercialization, it’s far down the track. Whereas in **continued>**

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“Get involved with the internship program at your company, so you can understand the student mindset.”

– Hans Schmitthenner



industry I know that there’s a direct connection between what I’m doing and a customer getting utility out of it.”

Of course with a change of setting comes a whole new system to comprehend. “You have to accept that when you leave industry you are like a child and have a lot to learn,” says Smith. “Get over your ego. It’s a humbling experience when you’re the new kid and everyone else is a life-long veteran.” Adds Schmitthenner, “I had reached the level of principal investigator in three companies, and I more or less had to start again at the bottom rung of the ladder in academia. This took considerable humility and patience.”

Advice for the jump

As you consider this life change, look for opportunities to demonstrate “the things that academia values: research, teaching, writing,” says Toyama. Since teaching is a big part of the academy, it is important to showcase your pedagogical expertise. Consider teaching courses at local universities, but in the absence of formal classroom experience, there are other ways to affirm “your ability to improve the skill set and knowledge of the people around you,” says Steel.

For example, seek out the opportunity to mentor interns, says Toyama, and treat them in the same way you would treat research protégés at a university. At Microsoft, “I managed junior researchers, similar to Ph.D. students,” he says. “I helped them become better researchers themselves.” Schmitthenner mentored co-op students from RIT in his lab at both AstraZeneca and Kodak. “Get involved with the internship program at your company,” he advises, “so you can understand the student mindset.”

And think innovatively about how to carry out your transition. If you launch academic collaborations while you are still working for a company, notes Smith, the enterprise might allow you to make a soft transition, in which you work part time for both. “Propose the idea to your boss...that you are eager to find a way that’s win-win,” such as an arrangement in which the company has access to university researchers and facilities, he suggests. But “reassure your employer that you know the bounds of confidentiality.”

Creating your own job might be the eventual way to go. “Look for the sweet spot where your background can fit,” says **Jim Hess**, a statistician who spent over 30 years in statistical consulting positions in multinational corporations such as DuPont. When he retired, he approached his alma mater, Southern Methodist University (SMU), about helping to grow its statistical consulting center. Now, as a visiting research professor, he is expanding the center’s commercial side to allow SMU students to work on projects provided by industry clients.

Of course, no sector transition is a walk in the park. “If your career in industry was not research-related, you’ll have to work to forge a research path,” admits Baglione. Additionally, industry activities may not translate well to search committees. “I knew that not all people in the academic world would understand what a chief scientific officer was or how that experience would help me as a faculty member,” says Fetrow. So as she tailored her curriculum vita, “I made parallels between my work in industry and academia. I very clearly articulated in my research plan what expertise I would bring from my company, and how I would turn it into a federally funded research program.”

But one thing is for sure: For those scientists and engineers who worked in industry and migrated to academia, the career benefits outweigh the hurdles. “Now that I made the transition, I’m extremely happy,” says Toyama. “It’s more than what I could have dreamed of for an academic job.” Adds Goldberg: “I was worried I’d be treated like a second-class citizen because I don’t do research. But that wasn’t the case. My colleagues valued my industry experience.”

Alaina G. Levine is a science careers writer based in Tucson, AZ.

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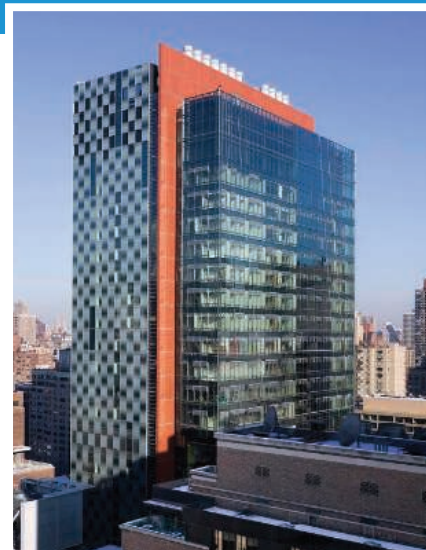
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Women, minorities, and members of other underrepresented groups are strongly encouraged to apply. Vanderbilt University is an Affirmative Action/Equal Opportunity Employer.



MULTIPLE FACULTY POSITIONS Department of Electrical and Systems Engineering

The School of Engineering and Applied Science at the University of Pennsylvania is growing its faculty by 33% over the next five years. As part of this initiative, the Department of Electrical and Systems Engineering is engaged in an aggressive, multi-year hiring effort for multiple tenure-track positions at all levels. Candidates must hold a Ph.D. in Electrical Engineering, Systems Engineering, or related area. The department seeks individuals with exceptional promise for, or proven record of, research achievement, who will take a position of international leadership in defining their field of study, and excel in undergraduate and graduate education. Leadership in cross-disciplinary and multi-disciplinary collaborations is of particular interest. We are interested in candidates in all areas that enhance our research strengths in:

1. **Nanodevices and nanosystems** (nanophotonics, nanoelectronics, MEMS/NEMS, power electronic devices, integrated devices and systems at nanoscale),
2. **Circuits and computer engineering** (analog and digital circuits, emerging circuit design, internet-of-things, computer engineering, embedded systems), and
3. **Information and decision systems** (data science, communications, information theory, control and optimization, robotics, signal processing, network science, markets and social systems).

Prospective candidates in all areas are strongly encouraged to address large scale societal problems in energy, transportation, health, economic and financial networks, critical infrastructure, and national security. We are especially interested in candidates whose interests are aligned with the school's strategic plan (www.seas.upenn.edu/PennEngineering2020).

Diversity candidates are strongly encouraged to apply. Interested persons should submit an online application at <http://www.es.eupenn.edu/faculty-positions> and include curriculum vitae, statement of research and teaching interests, and at least three references. Review of applications will begin on **December 1, 2015**.

The University of Pennsylvania is an Equal Opportunity Employer. Minorities/Women/Individuals with Disabilities/Veterans are encouraged to apply.

THE UNIVERSITY OF HONG KONG



Tenure-Track Associate Professor/Assistant Professor in the Department of Chemistry (2 posts)

Applications are invited for a tenure-track appointment as Associate Professor / Assistant Professor in Inorganic/Materials Chemistry [post (A) (Ref.: 201501148)] and in Computational Chemistry [post (B) (Ref.: 201501149)] in the Department of Chemistry, to commence on July 1, 2016 or as soon as possible thereafter. These posts are tenure-track positions with consideration for tenure during the second three-year appointment.

For post (A) (Ref.: 201501148), applicants should have a Ph.D. degree with a strong background and research record in the general area of inorganic/materials chemistry. **For post (B) (Ref.: 201501149)**, applicants should have a Ph.D. degree with a strong background and research record in the computational chemistry with research interests in material science, catalysis, energy or drug design. **For both posts**, the appointees are expected to develop original and independent research programs, and excel in both undergraduate and postgraduate teaching. A suitable start-up fund for research will be provided to the appointees. Information about the Department can be obtained at <http://www.chemistry.hku.hk>.

A globally competitive remuneration package commensurate with qualifications and experience will be offered. At current rates, salaries tax does not exceed 15% of gross income. The appointments will attract a contract-end gratuity and University contribution to a retirement benefit scheme, totalling up to 15% of basic salary, as well as annual leave, and medical benefits. Housing benefits will be provided as applicable.

Applicants should send a completed application form, together with an up-to-date C.V., a research proposal, and a statement of teaching philosophy by e-mail to scchem@hku.hk. They should also arrange for submission, to the same e-mail address as stated above, three reference letters from senior academics. Please indicate clearly the reference number and which level they wish to be considered for in the subject of the e-mail. Application forms (341/1111) can be downloaded at <http://www.hku.hk/apptunit/form-ext.doc>. Further particulars can be obtained at <http://jobs.hku.hk/>. **Closes November 30, 2015.**

The University thanks applicants for their interest, but advises that only candidates shortlisted for interviews will be notified of the application result.

The University is an equal opportunities employer and is committed to a No-Smoking Policy



Weill Cornell Medical College

Faculty Position in Chemistry/Chemical Biology

The **Department of Biochemistry of Weill Cornell Medical College (New York, NY)** seeks to hire a tenure track faculty member with research interests in synthetic chemistry and chemical biology. All faculty ranks will be considered. The applicant's laboratory will be in the newly opened Belfer Research Building on a floor that also houses the Tri-Institutional Therapeutics Discovery Institute (<https://www.triitdi.org/>) and other synthetic chemistry laboratories.

The new appointee will have a faculty appointment in the Department of Biochemistry (<http://www.cornellbiochem.org/>) and will be a member of the Graduate School of Medical Sciences and the Tri-Institutional Training Program in Chemical Biology (<http://chembio.triiprograms.org/>). The specific area of research is open with a preference for candidates interested in using chemical approaches to analyze cell biological and biochemical pathways and to study disease mechanisms.

Interested applicants should forward electronic versions of (1) a cover letter; (2) a Curriculum Vitae; (3) a brief overview of current and future research interests; and (4) the names and contact information of at least three referees. (Confidential inquiries can be considered without initial contact of referees.) Material should be sent to Frederick Maxfield, Chair of the Search Committee, at BiochemSearch@med.cornell.edu. Evaluation of applications will begin immediately and continue until the position is filled.



THE OHIO STATE UNIVERSITY

Columbus, Ohio

Assistant Professor Watershed Hydrologist

The Ohio State University is currently seeking an Assistant Professor, Watershed Hydrologist. This is a full-time, 9-month, tenure-track position in the School of Environment and Natural Resources (SENR).

The successful candidate will be expected to develop a nationally-recognized research program in watershed hydrology focused on hydrologic phenomena at the channel to catchment scale. Ideally, this scientist will integrate field observations and measurements with advanced techniques in hydrological modeling, geographical information systems (GIS), and/or remote sensing. Research areas of particular interest include (a) subsurface hydrologic processes, (b) surface water-subsurface water interactions (that can include, but are not limited to, nutrient fluxes, transport and fate of environmental pollutants, residence time/cycling), and (c) hydrological responses to climate change, land-cover change, and disturbances in agricultural and urban/suburban landscapes. Engineering hydrology (infrastructure analysis, design, construction, and operation) is outside of the scope of this position.

The incumbent will be expected to contribute to the SENR's teaching and advising programs in Environmental Science and to work with current faculty in related fields including aquatic ecology, terrestrial and fire ecology, soil science, water resource management, and environmental chemistry. The incumbent will be expected to develop an externally-funded research program, attract outstanding graduate students, and be committed to undergraduate education. Specific expectations and responsibilities include, but are not limited to:

- Develop an independent research program related to watershed hydrology with appropriate interdisciplinary alliances that includes a strong graduate student program.
- Teach graduate and undergraduate courses in area of expertise as well as within the Environmental Science curriculum. In particular, the incumbent will be expected to contribute to the undergraduate curriculum in both general water-quality management and watershed hydrology and to teach assigned undergraduate SENR service courses.
- Secure extramural funding and publish in refereed journals. The development of an interdisciplinary research program will be essential.
- Advise undergraduate students, including honors students, and assist with advising undergraduate student organizations.
- Work collaboratively with other University faculty/personnel, government agencies, non-government organizations, and citizen groups to advance the land grant mission of the University. Opportunities to work with the College's Field to Faucet as well as the University's Discovery Theme's initiatives may exist.
- Serve the University and professional communities through appropriate activities.

Earned Ph.D. with a strong academic background in hydrology. This position is designed to integrate and expand on existing research strengths in Aquatic Sciences. The ability to work with environmental social scientists is desirable. The successful candidate will demonstrate excellent verbal and written communication skills and a willingness and ability to work closely with others. Postdoctoral experience and previous involvement with state and federal agencies within the US or internationally are strongly encouraged. Demonstrated teaching scholarship and experience in mentoring members of underrepresented groups preferred.

Applications will be reviewed starting Dec. 18th and continue until a suitable candidate is identified. Applications must include the following: cover letter (1-2 pp); curriculum vitae; statements of research interests (1-2 pp), teaching philosophy (1 page), and multicultural experience and commitment to diversity/inclusion (1 page); and a list of four professional references with contact information.

Applicants should forward these materials via email as a consolidated pdf to:

Dr. Mažeika Sullivan
Watershed Hydrologist Search Committee Chair
School of Environment and Natural Resources,
The Ohio State University, 2021 Coffey Road, Columbus, Ohio 43210-1085
email address: sullivan.191@osu.edu

The Ohio State University is committed to establishing a culturally and intellectually diverse environment, and encouraging all members of our learning community to reach their full potential. We are responsive to dual-career families and strongly promote work-life balance to support our community members through a suite of institutionalized policies. We are an NSF Advance Institution and a member of the Ohio/Western Pennsylvania/West Virginia Higher Education Recruitment Consortium.

The Ohio State University is an equal opportunity employer. All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation or gender identity, national origin, disability status, or protected veteran status.



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You know that KU Leuven is a top-quality university. But are you also aware that we are one of the largest employers in Belgium with more than 13,000 dedicated professionals? An employer that promotes a climate of innovation, entrepreneurship and diversity? An employer with whom you can realise your ambitions?

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SCIENTIST WITH FOCUS ON THE ROLE OF INFLAMMATION AND THE IMMUNE RESPONSE IN THE FIELD OF REGENERATIVE MEDICINE

RESPONSIBILITIES

In the Department of Development and Regeneration

- You develop and monitor an excellent personal **research program** with focus on interactions between (stem) cells and the host inflammatory and immune system and the host stromal component.
- You ensure **high-quality education** broadly related to your research field, supervise master theses and act as a promoter of PhD students.
- Scientific, social and internal **services** are also part of your job.

REQUIREMENTS

- You have a degree in Biomedical Sciences or a related degree and a PHD whereby the subject of your doctoral thesis was related to the research field.
- The quality of your research is backed by publications in international journals, peer-reviewed publications, ...
- Teaching experience is welcome.

For more info about our job opening and applying online:

www.kuleuven.be/jobsite/scientist

Discover yourself. Begin with your job.



KU LEUVEN

KU Leuven pursues an equal opportunity and diversity policy.



Tenure Track Faculty Position in Regenerative Medicine and Cell Biology

Medical University of South Carolina,
Department of Regenerative Medicine
and Cell Biology

A tenure track faculty position at the Assistant or Associate Professor level is available for a researcher who uses cellular and/or molecular-genetic approaches to address fundamental aspects of human disease. Candidates interested in the study of cardiovascular or digestive disease using stem cells are particularly encouraged to apply. Competitive salary, laboratory space and start-up funds are available. Candidates at the Associate Professor level are expected to bring a vigorous research program with significant extramural funding. Current research strengths in the department include tissue engineering, pluripotent stem cell differentiation, cardiovascular development, digestive disease, and molecular biology of cell function. Research in the department is supported by several excellent core facilities specializing in imaging, genetically modified mice and rats, drug discovery, proteomics, genomics, and flow cytometry. A Ph.D. or M.D./Ph.D. degree (or equivalent), plus additional postdoctoral experience are required. To formally apply, please use this link: <http://www.jobs.musc.edu/postings/32091>. In addition, interested individuals should send a resume, research plans, and the names of three references to: **Dr. Stephen A. Duncan, Professor and Chair, Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, BSB 6th Floor, Room 657A, 173 Ashley Avenue, Charleston, SC 29425.**

The Medical University of South Carolina in Charleston was established in 1824 and is ranked in the upper quartile of all freestanding academic medical universities. The university has approximately 2,200 graduate and professional students that are supported by 2,000 faculty members. In 2014 total financial support topped \$217 million with \$100 million coming from the NIH. This includes funding that supports the NCI-designated Hollings Cancer Center as well as a Clinical and Translational Science Award.

MUSC is an Affirmative Action/Equal Opportunity Employer.



BioFrontiers Institute
UNIVERSITY OF COLORADO

BioFrontiers Institute Tenure-Track Faculty Position

The BioFrontiers Institute at the University of Colorado at Boulder invites applications for a tenure-track faculty position. The ideal applicant will establish an innovative interdisciplinary research program that synergizes with the Institute's core strengths in large datasets, networks and genomics, bioimaging from molecules to organisms, new therapeutic paradigms, and regenerative biology. Applicants in the area of chemical biology are particularly encouraged to apply.

BioFrontiers integrates faculty from ten departments to address significant problems in biology and medicine at the interface of the biological sciences with computer science, mathematics, physics, chemistry, and/or engineering (see <http://BioFrontiers.colorado.edu/about>). Faculty are expected to develop an internationally recognized research program combining these disciplines.

The tenure-track position is at the Assistant Professor level, although more senior candidates will also be considered. Candidates must have a Ph.D. and a demonstrated commitment to teaching at undergraduate and graduate levels. The successful candidate will hold the Marvin H. Caruthers Endowed Chair for Early Career Faculty for a period of four years.

Application materials are accepted electronically at <http://www.jobsatcu.com/postings/106683>. Review of applications will begin on **November 1, 2015** and will continue until the position is filled. The University of Colorado Boulder conducts background checks for all final applicants.

As an Equal Opportunity/Affirmative Action Employer, the University of Colorado is committed to diversity and equality in education and employment and sensitive to the needs of dual-career couples.



Tenure-Track Faculty Position Department of Microbiology and Physiological Systems

The Department of Microbiology and Physiological Systems at the University of Massachusetts Medical School (<http://www.umassmed.edu/>) invites applications for a **tenure-track faculty position** at the rank of **ASSISTANT PROFESSOR**. Depending on qualifications, candidates may be proposed for a more senior appointment at the rank of **ASSOCIATE** or **FULL PROFESSOR**. The Department is seeking candidates who use cross-disciplinary or systems-level approaches to solve problems in bacterial or viral infection including, but not limited to: molecular mechanisms of pathogenesis; cell biology of infection; analysis of complex microbial communities and their impact on the host; and host responses and adaptation to infection. Candidates will be expected to develop and maintain an innovative, externally funded research program. We offer generous support and a collaborative environment with opportunities for both basic and translational research. The position will be highly competitive with regard to start-up funds and salary. Review of applications will begin on September 1, 2015, and continue until the position is filled.

Applicants should submit a cover letter explaining their interest in the Department, a curriculum vitae that includes honors and publications, and a succinct research plan to <https://academicjobsonline.org/ajob/jobs/5742>. To expedite the review process, applicants should invite three individuals who are familiar with their work and potential for success to upload recommendation letters at the same web address.

UMass Medical School is committed to being an equal opportunity and affirmative action employer and recognizes the power of a diverse community. We encourage applications from protected veterans, individuals with disabilities and those with varied experiences, perspectives and backgrounds to consider UMass Medical School as their employer of choice.

Tenure-Track Assistant/Associate Professor Faculty Positions Department of Biology University of Miami, Florida

Developmental Biologist: Applications are invited from outstanding scholars engaged in addressing fundamental questions in any area of developmental biology. We welcome applications from candidates who would complement the existing strengths of our department and would be interested in contributing to cross campus collaborations with other academic units at the University of Miami. The College of Arts and Sciences has plans for a cross departmental initiative that will focus on biomaterials research, and candidates who would be able to contribute to this initiative are particularly encouraged to apply. Inquiries should be directed to the Search Chair at devbiosearch@bio.miami.edu.

Integrative Biologist: Applications are invited from outstanding scholars engaged in answering fundamental questions about the process of evolution using functional genomic approaches. To promote synergy within the department, candidates able to build on existing departmental strengths in areas that include neuroscience, behavior, tropical biology, ecology, and symbiosis, are especially encouraged to apply. Inquiries should be directed to the Search Chair at ibiosearch@bio.miami.edu.

To be eligible for these tenure-track appointments at the Assistant/Associate Professor level, successful candidates must hold a PhD, have postdoctoral experience, are expected to develop vigorous, externally funded research programs and to teach at both the undergraduate and graduate levels. More information about the Department and University can be found at <http://www.as.miami.edu/biology/>. Applicants should submit a cover letter describing interactions they foresee with existing university research programs, a curriculum vitae, two representative publications, a research statement, a teaching statement and the names of at least three references online to <http://www.as.miami.edu/biology/jobs/>. To receive full attention application materials must be received by **November 15, 2015**.

The University of Miami is an Equal Opportunity Employer, and Females / Minorities/Protected Veterans/Individuals with Disabilities are especially encouraged to apply. Applicants and employees are protected from discrimination based on certain categories protected by Federal law.

AAAS SCIENCE & TECHNOLOGY POLICY FELLOWSHIPS

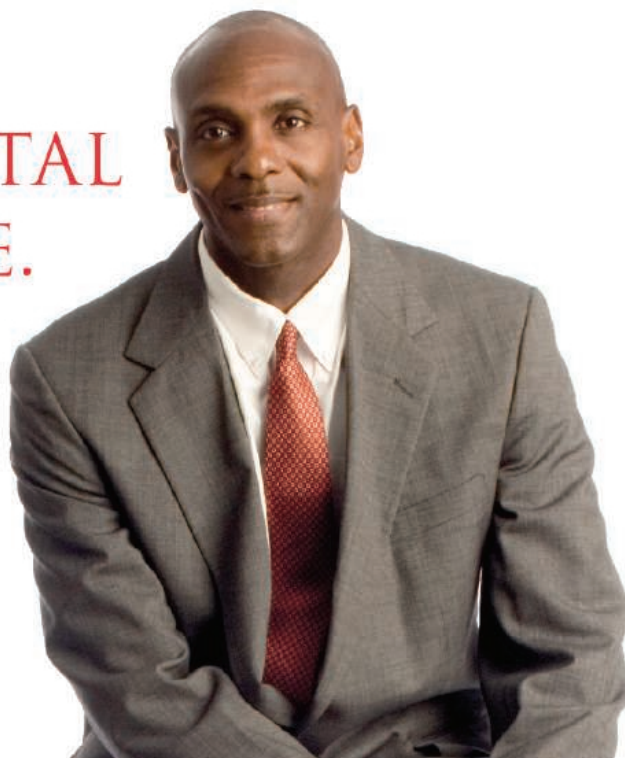


"I facilitated an agency knowledge management strategy."



I HAD A
MONUMENTAL
EXPERIENCE.
YOU CAN
TOO.

— Garrick Louis, PhD, Engineering & Public Policy; Executive Branch Fellow, Environmental Protection Agency



MAKE A DIFFERENCE.
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Apply your scientific analysis and technical knowledge to inform policy through assignments in the Legislative, Executive and Judicial Branches.

Stipends from
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\$100,000.
Applications due
November 1.

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Enhancing Policy, Transforming Careers





**Massachusetts
Institute of
Technology**

Assistant Professor Department of Chemical Engineering

The **MIT Department of Chemical Engineering** (<http://web.mit.edu/cheme/>) invites candidates for faculty positions starting July 2016 or thereafter. Appointment will be at the assistant or untenured associate professor level and will be located in Cambridge, MA. In special cases, a senior faculty appointment may be possible. Candidates must have a Ph.D. in chemical engineering or a related field by the start of their employment. Candidates with research and teaching interests in all areas relevant to the field of chemical engineering will be considered. The successful candidate is expected to advise students, and develop and teach chemical engineering courses at both undergraduate and graduate levels, as well as to develop a sponsored research program and be involved in service to MIT and the profession.

Interested candidates should submit application materials electronically at <https://chemefacsrch.mit.edu>. Each application must include: a curriculum vitae; the names and addresses of three or more references; a strategic statement of research interests; and a statement of teaching interests. It is the responsibility of the candidate to arrange for reference letters to be uploaded at <https://chemefacsrch.mit.edu/letters/>.

Please address questions to
ChemE-Search-Master@chemefacsrch.mit.edu.
Responses received by December 1, 2015, will be given priority.

With MIT's strong commitment to diversity in engineering education, research and practice, we especially encourage minorities and women to apply. MIT is an Equal Opportunity/Affirmative Action employer

<http://web.mit.edu>



Cleveland Clinic

Faculty Positions in Cancer Research Department of Cancer Biology, Lerner Research Institute (LRI)

The Department of Cancer Biology is seeking multiple cancer researchers at all levels (Assistant/Associate/Full Professor) with a focus in hematological/lymphoid malignancies, prostate or breast cancers with strong translational interests. The positions provide an exceptional opportunity to translate basic discoveries to the clinic through collaborative interactions with outstanding clinical programs.

The Department of Cancer Biology currently has 14 primary Faculty actively involved in basic and translational research programs investigating cancers of the brain, colon/rectum, kidney, and prostate, as well as hematologic malignancies. Research foci include cell signaling, DNA damage and repair, tumor suppressor genes, new targets, histone modification, cell cycle control, developmental therapeutics, drug resistance, invasion, metastasis, angiogenesis, tumor microenvironment, and the innate defense against viruses and cancer. The LRI offers over twenty Clinic-subsidized Core services including the Animal Tumor Core, Mass Spectrometry, Imaging and Confocal Microscopy, Genomics, and the Small Molecule Screening Core, allowing access to the latest state-of-the-art equipment.

To be considered, applicants must have M.D., M.D./Ph.D., or Ph.D. degrees and must have ongoing grant support and an accomplished research program in above-mentioned cancers. The successful applicants will be supported by generous start-up funds and joint appointment in Cleveland Clinic's Taussig Cancer Institute (part of the NCI designated Case Comprehensive Cancer Center) and Lerner College of Medicine. Candidates should submit curriculum vitae, summary of research interests, and three references, via e-mail to: **Colleen Johngrass (johngrc@ccf.org)**.

For further information see: <http://www.lerner.ccf.org/cancerbio/>

Cleveland Clinic is an Equal Opportunity/Affirmative Action Employer.



**College of
Osteopathic
Medicine**

BASIC SCIENCE FACULTY POSITIONS

To improve health care delivery in the Mississippi Delta region, **New York Institute of Technology College of Osteopathic Medicine (NYIT COM)** is opening a new medical school site at the Arkansas State University campus in Jonesboro, Arkansas. Basic science faculty positions at the **Assistant or Associate Professor** level will be recruited to the Department of Biomedical Sciences Arkansas site to meet teaching needs in the basic sciences. Initial recruitment areas will include **ANATOMY, BIOCHEMISTRY/GENETICS, NEUROSCIENCE, and PHYSIOLOGY**. The area of research focus is open. NYIT COM currently has active research in cardiovascular disease/heart failure, cancer biology, developmental anatomy, evolutionary morphology, renal physiology and development, neuroscience, microbiology, phylogenetic systematics, and vertebrate evolution. Successful candidates are expected to contribute to the medical school teaching effort and develop an active research program, with collaboration between researchers at both sites strongly encouraged. The medical school provides strong institutional support for both teaching and research activities. Jonesboro offers a wonderful Division I college town environment in close proximity to Memphis, TN.

The successful candidate will possess a Ph.D., D.O., M.D., or D.V.M. Two+ years of post-doctoral research experience by the start date and experience in medical education/teaching are strongly preferred. We offer institutionally supported faculty salaries, a competitive benefits package, and a professional environment designed to enhance career development. To apply, please e-mail cover letter and resume to: **Dr. A. Martin Gerdes** at agerdes@nyit.edu and **Dr. Jonathan Geisler** at jgeisler@nyit.edu, NYIT COM, PO Box 8000 Northern Blvd, Old Westbury, NY 11568-8000. Review of applications will begin immediately with a start date of **February 1, 2016**.

EOE M/F/D/V.



Tenure-Track Assistant Professor Position in Cardiovascular Science, Broadly Defined

The Department of Health Sciences in Boston University's College of Health and Rehabilitation Sciences: Sargent College is searching for a tenure-track faculty member at the Assistant Professor level in Cardiovascular Biology, broadly defined. The department has undergraduate programs in human physiology, health science, and nutrition, and graduate programs in human physiology and neurobiology (MS and PhD) and nutrition (MS DI). Faculty members have active research programs in muscle biology, cytoskeletal signaling, and neurobiology. Opportunities for collaborations also exist in other research institutes or departments on the Charles River and Medical School campuses. Applicants should have an earned doctorate in physiology, cell biology or a related field, including post-doctoral training. Applicants should have a strong scholarly record with research funding, or potential for funding from extramural sources, as well as a commitment to further develop departmental undergraduate and graduate programs. Review of applications will commence upon receipt, and will continue until the position is filled. Applicants should submit a letter of application, curriculum vitae, statement of research plans, a teaching statement and names of three individuals who can provide letters of reference to:

**Danuta Charland, Assistant to the Search Committee
Department of Health Sciences**

**College of Health and Rehabilitation Sciences: Sargent College
Boston University**

**635 Commonwealth Avenue, 4th floor
Boston, MA 02215**

E-mail to: charland@bu.edu

We are an Equal Opportunity Employer and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, national origin, disability status, protected veteran status, or any other characteristic protected by law.

We are a VEVRAA Federal Contractor.



Assistant Professors Neuroscience

The Institute of Neurobiology of the University of Puerto Rico Medical Sciences Campus seeks applicants for two tenure-track positions at the Assistant Professor level. We are conducting a broad search and will consider candidates who have a record of innovative research in the areas of neuroscience or neurophysiology. Researchers who use invertebrate model and non-model systems are encouraged to apply. The candidate should have a doctoral degree, a minimum of two years postdoctoral training, and research productivity commensurate with experience. The successful candidate will be expected to establish an active, externally-funded research program. Fluency in Spanish is desirable but not essential. This is a full-time research position that carries no teaching obligations; however, opportunities to obtain an academic appointment in a relevant department of the School of Medicine are available. In addition, 100% of the base salary is guaranteed by the institution and competitive researchers have the opportunity to receive significant bonuses and tax breaks. Neuroscience has been targeted by UPR as a special area of growth, and this is reflected in both infrastructure investment and faculty hiring. The successful candidate will receive a competitive start-up package and laboratory space at the Institute of Neurobiology, in the heart of historic Old San Juan.

Assemble a curriculum vitae, a description of research interests, a plan for obtaining funding, the names and contact information of three referees, and reprints of two significant publications into a single pdf document and email it to: **Dr. Joshua Rosenthal** (rosenthal.joshua@gmail.com). In order to receive full consideration, applications should be received by **December 15, 2015**. The start date for these positions will be July 2016. For more information please contact **Dr. Joshua Rosenthal** at the above email.

UPR-MS is an Equal Opportunity/Affirmative Action Employer.

Academic Career Opportunities



Position Title: Faculty Position

Req # 02788

The University of Chicago's Department of Biochemistry and Molecular Biology seeks candidates committed to the cryo-electron microscopy of macromolecules. Appointment will be made at the rank of Assistant Professor, Associate Professor, or Professor, depending on qualifications. A PhD or MD (or foreign equivalents) along with relevant postdoctoral experience are required. Applications for all ranks must include a curriculum vitae, a research statement that sets forth plans for future research in the cryo-electron microscopy of macromolecules, and a teaching statement. These materials must be uploaded to the University of Chicago's Academic Career Opportunities website at academiccareers.uchicago.edu/applicants/Central?quickFind=54276. Applicants for a position at the rank of Assistant Professor should make arrangements for the submission to tarnisha@uchicago.edu of up to three letters of reference. Review of applications will continue until the position is filled.

All qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, age, protected veteran status or status as an individual with disability.

The University of Chicago is an Affirmative Action/Equal Opportunity/Disabled/ Veterans Employer.

Job seekers in need of a reasonable accommodation to complete the application process should call **773-702-5671** or email **ACOppAdministrator@uchicago.edu** with their request.



Massachusetts
Institute of
Technology

Come work with us!

Tenure Track Faculty

The Department of Brain & Cognitive Sciences (BCS) (<http://bcs.mit.edu>) at MIT is looking to hire up to five (5) tenure-track faculty at the assistant professor level. Affiliations with the Picower Institute for Learning & Memory and the McGovern Institute for Brain Research are possible. We are most excited about candidates who work in one or more of the following four (4) areas:

i. Computational and theoretical approaches to neuroscience and cognition. Possible areas of focus include but are not limited to: statistics and data science, neural circuits, neural population representations and transformations, and cognitive processes. Candidates with the ability to build bridges across empirical domains are especially attractive. An affiliation with Electrical Engineering and Computer Science (EECS), the Computer Science and Artificial Intelligence Laboratory (CSAIL), Institute for Data, Systems, and Society (IDSS), or other allied departments is possible.

ii. Systems neuroscience in non-human animals. The ideal candidate would be driven by computational questions and ideas from human cognition, with the goal of reverse engineering the underlying neural representations and processes using tools that allow access to multiple brain regions.

iii. Cognitive neuroscience in humans, especially if the candidate's work bridges levels of analysis using a variety of methods including MRI, MEG, fMRI, theoretical modeling, genetics and reverse engineering approaches.

iv. Human cognition using behavioral methods, especially in the areas of language and/or cognitive development.

Successful applicants are expected to develop and lead independent, internationally competitive research programs and to share in our commitment to excellence in undergraduate and graduate education by teaching courses and mentoring graduate and undergraduate students. PhD must be completed by start day of employment and some postdoctoral training is preferred.

Please submit application materials – cover letter, CV, statement of research and teaching interests and representative reprints – online at <https://academicjobsonline.org/ajob/jobs/5972>. Please state research area in cover letter. To help direct the application, applicants should indicate which of the four areas listed above is their main research area by answering the mandatory questions included in the application. In addition, please arrange to have three letters of recommendation submitted online. Review of applications will begin on October 31, 2015.

MIT is an affirmative action employer, and we encourage applications from women and underrepresented minorities.

<http://web.mit.edu>

Keck School of Medicine of USC

Emerging Pathogens TENURE-TRACK FACULTY POSITIONS

Department of Molecular Microbiology and Immunology
USC Institute of Emerging Pathogens and Immune Diseases
Keck School of Medicine
University of Southern California
Los Angeles, California

The Department of Molecular Microbiology and Immunology at the Keck School of Medicine of the University of Southern California in Los Angeles, California, has an ongoing expansion to build upon existing strengths in Microbiology, Virology, and Immunology.

The Department invites applicants for tenure-track Assistant and/or Associate Professor positions with a specific research emphasis on emerging pathogens and immune responses. We are especially interested in candidates whose research addresses biodefense pathogenesis-related, trans-disciplinary, and translational research topics. Creative scientists with a record of achievement and commitment to excellence in both research and teaching are encouraged to apply. Successful candidates will receive generous start-up packages and laboratory space along with access to a new Biosafety Laboratory 3 facility. The Keck School of Medicine has strong research programs in Cancer, Genomics, Immunology, Stem Cells, Neurobiology, and Virology.

Applicants should submit a letter of application, Curriculum Vitae, a statement of current and future research plans, and three letters of recommendation. Please complete faculty application through the USC job website at www.jobs.usc.edu with USC Job Code **P01456930**.

USC values diversity and is committed to equal opportunity in employment. Women and men, and members of all racial and ethnic groups are encouraged to apply.



UNIVERSITY OF
TORONTO

Assistant Professor - Systems Biology

The Department of Cell & Systems Biology at the University of Toronto invites applications for a tenure-stream appointment in Systems Biology at the rank of Assistant Professor beginning July 1, 2016.

We will consider all outstanding applicants with a track record and demonstrated excellence in applying systems biology and/or synthetic biology approaches to address basic biological questions that will complement the strengths of the Department in genomics, plant and microbial biology, cell and developmental biology, or neuroscience. U of T is an outstanding world-class scientific environment with a highly interactive community of researchers.

The successful candidate must have a Ph.D. in Biology or related field (e.g., Systems Biology, Synthetic Biology, Molecular & Cellular Biology, Genome Biology, Genetics, Neuroscience, etc.), and substantial postdoctoral experience. The candidate will be expected to pursue a vigorous internationally recognized research program. Evidence of excellence in research will be demonstrated by publications in top ranked and field relevant academic journals, presentations at significant conferences, awards and accolades, and strong endorsements by referees. In addition, we expect a strong commitment to excellence in teaching and mentoring of students. Salary will be commensurate with qualifications and experience. All qualified candidates are invited to apply online at <http://uoft.me/1236>. Applications should include a cover letter, curriculum vitae, statement of teaching philosophy and experience, and a statement outlining current and future research interests. If you have questions contact csbsearch@utoronto.ca.

Applicants should ask at least 3 referees to send letters directly to the Chair at csbsearch@utoronto.ca by the closing date, **November 30, 2015**. Please ensure the candidate's name is included in the subject line, that letters are on department letterhead, and signed by the referee.

For more information about the Department of Cell & Systems Biology, visit: <http://www.csb.utoronto.ca/>.

The University of Toronto is strongly committed to diversity within its community and especially welcomes applications from visible minority group members, women, Aboriginal persons, persons with disabilities, members of sexual minority groups, and others who may contribute to the further diversification of ideas.

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.



PRINCETON
UNIVERSITY

ASSISTANT PROFESSOR, EVOLUTIONARY GENOMICS Faculty Position

Princeton University's Department of Ecology and Evolutionary Biology, in partnership with the Lewis Sigler Institute for Integrative Genomics, is seeking candidates for a tenure track faculty position at the Assistant Professor level in the area of genomics and evolution.

We are interested in scientists working at the intersection of functional genomics and evolutionary biology, who use approaches that include the analysis of large data sets. The successful candidate will address fundamental questions in evolutionary and population biology with the aim of offering novel conceptual advances and strengthen intellectual bridges between evolutionary and systems biology. They will also have the opportunity to interact with a diverse and interdisciplinary group of like-minded faculty including members from Ecology and Evolutionary Biology, Computer Science, Molecular Biology, the Lewis Sigler Institute for Integrative Genomics, the Princeton Neuroscience Institute and the newly-formed Center for Statistics and Machine Learning. More broadly, we seek colleagues who will enthusiastically contribute to a climate of interdisciplinary collaboration, excellence and diversity.

Applicants should write a vision statement, no longer than two pages, that outlines one or more major unsolved problems in their field and how they plan to address them. In this respect, the vision statement should go beyond a summary of the applicant's prior and current research. Applications, including the vision statement, curriculum vitae, three reprints, and contact information for three references can be submitted online via <http://jobs.princeton.edu>, Req #1500823. Screening of applications will begin **November 15, 2015**.

Princeton University is an Equal Opportunity Employer and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, disability status, protected veteran status, or any other characteristic protected by law. This position is subject to the University's background check policy.



PRINCETON
UNIVERSITY

ASSISTANT PROFESSOR: ECOSYSTEM BIOLOGY, COMMUNITY ECOLOGY, OR GLOBAL CHANGE BIOLOGY

Princeton University's Department of Ecology and Evolutionary Biology is seeking candidates for a tenure-track faculty position in the areas of ecosystem biology, community ecology, and/or global change biology. We are interested in scientists who address fundamental questions with the goal of offering novel conceptual advances, and whose work is relevant to contemporary environmental problems. The successful applicant will have the opportunity to interact with a diverse and interdisciplinary group of like-minded faculty members in several departments and in the Princeton Environmental Institute.

Applicants should write a vision statement, no longer than two pages, that outlines one or more major unsolved problems in their field and how they plan to address them. In this respect, the vision statement should go beyond a summary of the applicant's prior and current research.

Applications, including the vision statement, curriculum vitae, three reprints, and contact information for three references can be submitted online via <http://jobs.princeton.edu>, Req #1500822. Screening of applications will begin **November 15, 2015**.

Princeton University is an Equal Opportunity Employer and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, disability status, protected veteran status, or any other characteristic protected by law. This position is subject to the University's background check policy.



Faculty Position in the Life Sciences

The Stowers Institute for Medical Research invites innovative young scientists in the Life Sciences to submit applications for a faculty position. We anticipate making an appointment in 2016 at the rank of Assistant Investigator. Research programs of interest include, but are not limited to: biochemistry, quantitative biology, neuroscience, developmental and cell biology, genomics, stem cell biology, regenerative biology and epigenetics. Our interests encompass a broad gamut of experimental organisms and approaches. The successful candidate is expected to develop a groundbreaking, innovative and independent research program and will benefit from and complement the Institute's existing strengths in genetics and epigenetics, cell and chromosome biology, stem cells and regenerative biology, developmental biology and evolution, and biochemistry and neuroscience.

The position is fully funded throughout the candidate's appointment. This includes \$600,000 per year for full salary support and research funding, in addition to start-up funds and ongoing needs for equipment. The initial appointment is for 6 years and is then subject to renewal every 6 to 7 years. In total, the package for a junior position is more than \$3.6 million over the first term and increases significantly after promotion. In addition, investigators may take advantage of exceptional core facilities and technology centers staffed by over 100 scientists. Stowers investigators have multiple opportunities to be involved in the Institute's Graduate School program.

Candidates must have a Ph.D. or equivalent degree and postdoctoral experience demonstrating innovation and excellence in their field. Candidates will be expected to possess a long-term vision of their scientific interests, to establish a vigorous and innovative research program, and to actively contribute to the Institute's mission and collegiality.

Deadline for applications is **November 1, 2015**. Applicants should submit a cover letter, a CV, a research plan and vision statement, and arrange for the submission of three letters of reference through our application page at: <http://www.stowers.org/facultysearch>.

Questions should be directed to the Search Committee Chair, Dr. Alejandro Sánchez Alvarado (facultysearch@stowers.org).

The Stowers Institute for Medical Research is proud to be an Equal Opportunity Employer. All qualified applicants will be afforded equal opportunity regardless of race, creed, color, religion, gender, sexual orientation, pregnancy, national origin, age, disability, military status, or any other status protected by law.



Dean of the School of Engineering and Applied Science

Princeton University seeks an exceptional academic leader to serve as its next Dean of the School of Engineering and Applied Science (SEAS). Appointed by President Christopher Eisgruber and the Board of Trustees, and reporting to Provost David Lee, the Dean is responsible for the quality, conduct, administration, planning, and development of the school's teaching programs and research activities.

The successful candidate will possess an outstanding record of accomplishment in research, teaching and administration, and will demonstrate uncompromising commitment to academic and educational excellence, to the highest ethical standards, and to the creation and support of a vibrant and broadly diverse community of faculty, students and staff across SEAS.

The next dean will take office at a pivotal moment in the School's history. The School has completed a strategic planning process that identified key priorities, and the University's president has emphasized the importance of engineering to Princeton's future. One of the dean's most important assignments will be to build on the foundation laid by the strategic planning process, as well as the opportunities that emerge going forward.

Princeton's School of Engineering and Applied Science is home to an eminent faculty of more than 140 members in six academic departments and four major centers, and an exceptional student body numbering more than 1,000 undergraduate and 500 graduate students. The current faculty includes 19 members of the National Academy of Engineering and 8 members of the National Academy of Sciences. Annual research expenditures exceed \$70 million. The school is a diverse, vibrant and highly collaborative community of teaching, learning, innovation and exploration, whose members aim to advance the frontiers of knowledge across the engineering disciplines and to apply this knowledge to the benefit and advancement of society.

Applications should include a letter of interest and a CV, and should be submitted online to <http://jobs.princeton.edu>.

Nominations may be sent to seassearch@princeton.edu.

To receive full consideration, nominations and applications should be received by **November 13, 2015**.

Princeton University is an Equal Opportunity Employer and all qualified applicants will receive consideration for employment without regard to race, color, religion, sex, sexual orientation, gender identity, national origin, disability status, protected veteran status, or any other characteristic protected by law.



FACULTY POSITION ANNOUNCEMENT

Associate/Full Professor of Postharvest Physiologist Department of Plant Science at the University of California, Davis

The University of California at Davis is pleased to announce the recruitment for a tenure-track faculty position in Postharvest Physiologist. The successful candidate will join the Department of Plant Sciences in the College of Agricultural and Environmental Sciences at the rank of Associate/Full Professor.

Qualifications: Ph.D. in plant physiology, biology, horticulture, biochemistry or related discipline with experience and documented central focus in postharvest plant physiology. International development experience is desirable.

Salary: Commensurate with qualifications and experience.

To Apply: The complete position description and application instructions can be viewed at apptrkr.com/680188. For questions regarding the application, processes please send e-mail to kgeer@ucdavis.edu. Review of applications will begin November 15, 2015. The position will remain open until filled.

UC Davis is an affirmative action/equal employment opportunity employer and is dedicated to recruiting a diverse faculty community. We welcome all qualified applicants to apply, including women, minorities, veterans, and individuals with disabilities.



Junior Faculty Position

The Clinical and Translational Science Center at the University of New Mexico invites applicants for a junior faculty member for our Mentored Career Development (KL2) Scholar Program at the Assistant Professor level in the tenure or flex track beginning in the Spring of 2016. Individuals with a MD, PhD, MD/PhD or equivalent degree engaged in all types of infectious, renal, and metabolic (including obesity and diabetes) diseases are encouraged to apply. The successful Scholar candidate will have 75% of their effort committed to their research and 25% committed to other duties and will be able to become a faculty member in one of the following departments in the School of Medicine: Internal Medicine, Molecular Genetics & Microbiology, Biochemistry & Molecular Biology, Epidemiology, or Psychiatry.

For complete details or to apply, visit <https://unmjobs.unm.edu>. Reference posting number: **#0832075**. For best consideration, apply by: **November 15, 2015**. This position will remain open until filled. UNM's confidentiality policy, which includes information about public disclosure of documents submitted by applicants, is located at <https://www.unm.edu/~brpm/r67.htm>

UNM is an EEO/AA Employer.



**DEVELOPMENTAL BIOLOGIST
ASSISTANT PROFESSOR (TENURE-TRACK)**
DEPARTMENT OF BIOLOGICAL SCIENCES
COLLEGE OF SCIENCE

The Department of Biological Sciences at Louisiana State University (LSU) invites applications for a tenure-track position at the level of Assistant Professor. The successful candidate will be expected to establish and maintain a vigorous, extramurally funded research program in the area of eukaryotic developmental biology. Biological Sciences is a large and dynamic department with research ranging across all levels of biological organization from molecules to ecosystems. Preference will be given to individuals with an interest and record of achievement in research that complement the departmental strengths. Information about the department is available at <http://www.lsu.edu/science/biosci/>. The successful candidate will also contribute to undergraduate/graduate teaching. Applicants must have a Ph.D. in a Biological Science or related field and a successful track record of independent research.

An offer of employment is contingent on a satisfactory pre-employment background check. Application deadline is November 30, 2015, or until a candidate is selected. Apply online for position #001309 at: <https://lsusystemcareers.lsu.edu>, where you can view a more detailed ad and will upload a cover letter and application materials including a statement on how you would help LSU attain its goals as stated in its Flagship 2020 agenda, <http://www.lsu.edu/flagshipagenda/goals2020.shtml>.

Quick link at ad URL: <https://lsusystemcareers.lsu.edu/applicants/Central?quickFind=59966>

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EQUAL OPPORTUNITY/EQUAL ACCESS EMPLOYER*



**VASCULAR PLANT SYSTEMATICS
ASSISTANT PROFESSOR (TENURE-TRACK)
AND HERBARIA DIRECTOR**
BIOLOGICAL SCIENCES, COLLEGE OF SCIENCE

Louisiana State University Department of Biological Sciences invites applications for a tenure-track Assistant Professor and Herbaria Director position (see <http://www.herbarium.lsu.edu>). Exceptional candidates at the Associate Professor level will also be considered.

Responsibilities: Develop and maintain a vigorous, extramurally funded, specimen-based research program in vascular plant systematics; teach undergraduate and graduate classes; curate and further develop LSU's Herbaria collections with support of a recently enhanced endowment and a collections manager; and participate in extracurricular activities at LSU.

Required Qualifications: Ph.D. degree in plant biology or related area; a successful record of independent research in vascular plant systematics; and experience in collecting, managing, and using herbarium specimens in research.

Preferred Qualifications: Postdoctoral experience preferred.

An offer of employment is contingent on a satisfactory pre-employment background check. Application deadline is November 30, 2015, or until a candidate is selected. Anticipated start date is August 2016. We encourage applications from women and minorities. Apply online for position #029339 at www.lsu.edu/flagshipagenda, where application materials are uploaded, including a statement of how you would help LSU attain the goals stated in Flagship 2020, <http://www.lsu.edu/flagshipagenda>.

Quick link at ad URL: <https://lsusystemcareers.lsu.edu/applicants/Central?quickFind=59946>

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**BIOENERGY BIOCHEMIST
ASSISTANT PROFESSOR (TENURE-TRACK)**
DEPARTMENT OF BIOLOGICAL SCIENCES
COLLEGE OF SCIENCE

LSU seeks an Assistant Professor for a tenure-track faculty position. The successful candidate will be expected to establish and maintain a vigorous, extramurally funded research program that emphasizes bioenergy resources. Research topics can include, but are not limited to, bioelectricity, microbial fuel cells, and carbohydrate metabolism or photosynthetic mechanisms in plants or microorganisms. Biological Sciences is a large and dynamic department, with research ranging across all levels of biological organization from molecules to ecosystems. The successful candidate will complement these strengths and contribute to undergraduate and graduate teaching.

Required Qualifications: Ph.D. in Biochemistry, Microbiology, Biophysics or related field; successful track record of independent research.

Preferred Qualifications: Postdoctoral experience preferred.

An offer of employment is contingent on a satisfactory pre-employment background check. Application deadline is November 30, 2015, or until a candidate is selected. Anticipated start date is August 2016. We encourage applications from women and minorities. Apply online for position #038989 at: <https://lsusystemcareers.lsu.edu> where you will upload a cover letter and application materials including a statement on how you would help LSU attain its goals as stated in its Flagship 2020 agenda, <http://www.lsu.edu/flagshipagenda/goals2020>.

Quick link at ad URL: <https://lsusystemcareers.lsu.edu/applicants/Central?quickFind=59945>

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VANDERBILT  UNIVERSITY
MEDICAL CENTER

DIRECTOR OF RESEARCH

**Osher Center for Integrative Medicine at
Vanderbilt University Medical Center**

Applicants are sought for the position of Director of Research of the Osher Center for Integrative Medicine in the Department of Physical Medicine and Rehabilitation at Vanderbilt University. Competitive applicants will have an academic rank of Associate Professor or Professor and a record of sustained funding and scientific publications and may qualify for an endowed chair. We seek individuals capable of building and leading a research team, and engendering interdisciplinary collaborations. Applications from either Clinician Scientists or PhD Scientists with interest in integrative medicine and mind-body practices are welcome. A completed application should include a letter of interest, a curriculum vitae and a list of references. Send applications to Walter R. Frontera, MD, PhD; Professor and Chair; Department of PM&R, 2201 Children's Way, Suite 1318, Nashville, TN 37212. Tel: 615-322-7574; email walter.frontera@vanderbilt.edu.

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Vanderbilt University School of Medicine is an Equal Opportunity/Affirmative Action Employer.



FACULTY POSITION IN CELL AND DEVELOPMENTAL BIOLOGY THE UNIVERSITY OF CONNECTICUT

The Department of Molecular and Cell Biology in the College of Liberal Arts and Sciences at the University of Connecticut seek applicants for a nine-month, tenure-track faculty position at the assistant professor level at the Storrs campus starting 8/23/16 (Job Opening ID # 2016164). Although candidates working in any area of cell and developmental biology may apply, we are particularly interested in virus-host cell interactions and the immune response to viral infection, vertebrate development, stem cell biology, computational cell biology, cytokinesis and cytoskeletal dynamics.

For details on this position, qualifications, and application instructions please visit www.jobs.uconn.edu.

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To learn more, visit
aaas.org/plusyou/sciencecareers



THE UNIVERSITY OF TEXAS

MD Anderson Cancer Center

Making Cancer History®

The Department of Epigenetics and Molecular Carcinogenesis at The University of Texas MD Anderson Cancer Center, Science Park, <http://sciencepark.mdanderson.org>, seeks applications for a term tenure-track position at the Assistant Professor level. Research in the department encompasses cellular, genetic and epigenetic mechanisms important to oncogenesis and tumor suppression, stem cell pluripotency, immune cell function, cell death mechanisms, and genome integrity. We seek individuals working in these areas, with preference for strong bioinformatics expertise. Candidates must be committed to working in a highly collaborative, interdisciplinary environment. The successful candidate will be expected to develop and maintain an internationally recognized and competitively funded research program, and to participate in graduate student training. The department has outstanding core support services, including facilities for analysis of genetically engineered mouse models, imaging, and next generation sequencing. The department is located in a pine-oak forest near the city of Austin, TX, but is closely aligned with the main MD Anderson campus in Houston. MD Anderson offers outstanding research facilities, startup packages, and faculty benefits. Required qualifications include a Ph.D. (or equivalent), postdoctoral or independent scholarly research experience, and a strong publication record.

Candidates should submit a cover letter outlining the relevance of their research experience and interests to the position description, statement of current and proposed research interests, names and contact information for three references, and a CV as a single PDF to sciencepark@mdanderson.org by January 1, 2016.

MD Anderson is an equal opportunity employer and does not discriminate on the basis of race, color, religion, age, national origin, sex, sexual orientation, gender identity/expression, disability, veteran status, genetic information or any other basis protected by federal, state or local laws, unless such distinction is required by law. All positions at The University of Texas MD Anderson Cancer Center are security sensitive and subject to examination of criminal history record information. Smoke-free and drug-free environment.

Basic Science/ Epigenetics and Molecular Carcinogenesis Assistant Professor, Term Tenure Track

Email: sciencepark@mdanderson.org

UIC COLLEGE OF UNIVERSITY OF ILLINOIS AT CHICAGO MEDICINE

Faculty Positions in Host-Pathogen Interactions Department of Microbiology and Immunology

We are seeking outstanding candidates for **TENURE-TRACK** faculty positions at the Assistant or Associate Professor level in the **Department of Microbiology and Immunology** at University of Illinois at Chicago (UIC) College of Medicine in Chicago. Ideal applicants will be creative and collaborative scientists pursuing fundamental/translational aspects of bacterial or viral pathogenesis and host response to infection and using state-of-the-art approaches that combine genomics, cell biology, genetics, bioinformatics, biochemistry, and animal models of infection. We especially encourage applicants with innovative new perspectives and research interests that embrace interdisciplinary approaches. UIC offers an intellectually exciting, collegial, and supportive environment with robust graduate, MSTP, and post-doctoral programs and extensive opportunities for interdepartmental collaboration. Resources include outstanding core facilities for DNA/RNA sequencing and genomics, imaging, mass spectrometry, flow cytometry, structural analyses, and automated screening. Additional information can be found at chicago.medicine.uic.edu/departments_microbiology_immunology. Applicants should be committed to teaching graduate and professional students and establishing a vigorous, cutting-edge, externally funded research program. Requirements include a Ph.D. or equivalent degree in a relevant discipline, postdoctoral training, and a strong publication record. The College is part of a larger University of Illinois campus situated in Chicago, a uniquely cosmopolitan and global city.

For fullest consideration, individuals should submit an application comprising their curriculum vitae and research proposal (no more than 3 pages) describing plans for an independent research program by **November 1, 2015** at the following link: <https://jobs.uic.edu/job-board/job-details?jobID=55027&job=assistant-associate-professor-tenure-track>; applications thereafter will be considered on a rolling basis. Three letters of recommendation should be sent separately to Ms. Kate Campbell at kcamp@uic.edu. For questions related to the position please contact: **Nancy Freitag, PhD., Chair, Microbiology & Immunology Search Committee, University of Illinois at Chicago, Dept. of Microbiology and Immunology M/C 790, 835 S. Wolcott Ave., Chicago, IL 60612; Phone: 312-355-4903; Email: nfreitag@uic.edu**. For technical assistance please contact *Email: kcamp@uic.edu*.

*The University of Illinois at Chicago is an Equal Opportunity, Affirmative Action Employer.
Minorities, women, veterans and individuals with disabilities are encouraged to apply.*



Developmental Biology Faculty Position: Caltech, Pasadena, CA, United States

The California Institute of Technology invites applications for a tenure-track assistant professor position in the Division of Biology and Biological Engineering. We are interested in candidates who will approach fundamental questions regarding the cellular and molecular basis of development of multicellular organisms, with emphasis on systems level approaches. Relevant research areas include but are not limited to gene network and circuit design problems in developmental biology, mechanistic studies of morphogenesis, and evolution of developmental gene regulatory networks.

Candidates with strong commitments to research and teaching excellence are encouraged to apply. Initial appointments at the assistant professor level are for four years, and they are contingent upon completion of the Ph.D. degree.

Please submit on-line applications at: <http://bbe.caltech.edu/Positions>, and include a brief cover letter; curriculum vitae; relevant publications, a description of proposed research; and a statement of teaching interests. Applicants should arrange to have four reference letters uploaded. The **application deadline is November 1, 2015**. Applicants must be prepared to **attend a recruiting symposium at Caltech on December 15-16, 2015**, where they will present their research and future directions.

EOE of Minorities/Females/Protected Vets/Disability



UNIVERSITY OF MINNESOTA

Faculty Position in Immunology

The Department of Microbiology and Immunology at the University of Minnesota Medical School invites applications for a faculty position to be filled at the tenure-track Assistant Professor level.

We seek an outstanding scientist who will establish a competitive extramurally funded research program that focuses on any area of immunology that would complement or expand existing institutional strengths. The position offers exceptional startup support, a dynamic intellectual environment, state-of-the-art facilities, a competitive salary, and quality research space within the Center for Immunology (<http://www.immunology.umn.edu>). Additional information about the Department of Microbiology and Immunology, affiliated institutes and centers, and the graduate training program, can be found at <http://www.microbiology.med.umn.edu>.

Minimum qualifications: Ph.D., M.D., or equivalent in a relevant field of study, plus applicable postdoctoral or faculty experience. To apply, please upload a curriculum vitae and concise summary of current and planned research in response to job opening ID 304866 at <http://www1.umn.edu/ohr/employment>. Please also arrange to have 3 letters of recommendation sent to microbiology@umn.edu or **Immunology Search Committee, Department of Microbiology and Immunology, University of Minnesota, MMC 196, 420 Delaware Street S.E., Minneapolis, MN 55455**. Review of applications will begin **November 15, 2015** and continue until the position is filled.

The University of Minnesota provides equal access to and opportunity in its programs, facilities, and employment without regard to race, color, creed, religion, national origin, gender, age, marital status, disability, public assistance status, veteran status, sexual orientation, gender identity, or gender expression. To learn more about diversity at the U: <http://diversity.umn.edu>

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暨南大學
JINAN UNIVERSITY

百年暨大，誠聘英才
攜手暨大，共創未來

Jinan University implements Jinan Double Hundred Talents Plan to recruit global talents

Jinan University, first university of overseas Chinese founded by the state, with the largest foreign student among the country currently, is the national "211 Project" of key comprehensive university directly under the guidance of Overseas Chinese Affairs Office of State Council. In June 2015, Jinan University was listed as high-level university construction by Guangdong provincial Party Committee and the provincial government.

To further strengthen the support for the introduction of outstanding personnel and training, and decides to implement the "Jinan Double Hundred Talents Plan". According to the school discipline construction and innovative platform construction, Jinan University's five-year plan focuses on the introduction and cultivation of about 30 academicians, entrants in Thousand Talents Program, Cheung Kong Scholars, winners of National Science Fund for Outstanding Young Scholars and some other greater discipline leaders having influence in international and domestic field; about 50 talents in Thousand Talents Program, outstanding winners of National Science Fund for excellent Young Scholars, outstanding young scholars, Zhujiang scholars and outstanding young academic leaders emerging in the relevant academic fields, about 120 outstanding young academic backbone having strong academic developmental potential and overseas academic experience. "Jinan Double Hundred Talents Plan" implements principles of the strategy of open recruitment at home and abroad, the focused stimulation, objective assessment and management of the duration of employment, which is divided into three levels: the leading, the outstanding, and the top-notch.

I. Basic requirements for candidates

a. Leading talent: take honor in Thousand Talent Program, Cheung Kong Scholars Program, National Science Fund for Outstanding Young Scholars program or other equivalent level of academic status. Scientific & medical: age of 50 and below; Humanities & social sciences: age of 55 and below.

b. Outstanding talent: take honor in Thousand Talents program, National Science Fund for Outstanding Young Scholars Program, the Youth of the Yangtze Program, Zhujiang Scholars Program, special branch program of leading talent of Guangdong Province and other equivalent level of academic status. Scientific & medical: age of 42 and below; Humanities & social sciences: age of 47 and below.

c. Top-notch personnel: selected in the defense and conference assessment of Thousand Talents program, National Science Fund for Outstanding Young Scholars program, Outstanding Young Scholars Program, or other equivalent level of academic status. Scientific & medical: age of 35 and below; Humanities & social sciences: age of 40 and below.

II. Treatments provided

Jinan University provides selected candidates of "Jinan Double Hundred Talents Plan" with preferential plan in doctoral enrollment, post-doctoral enrollment and research assistant, and favorable living and working conditions and competitive benefits; the call and direct interviews to school is available.

III. Candidate material

a. A resume

b. Assumed research projects of nearly five years, published papers (specify the collection situation, journal impact factor and the number of papers he cited of SCI, EI, SSCI, CSSCI), the list of award-winning achievements;

c. Copy of Degree Certificates/Diploma, all research projects, awards and patents;

d. The full text of 5 representative papers;

e. Copy of certificate and incumbents certificate of holding an important position in the foreign office or in the domestic one;

f. Work plan after coming to school.

Advertisement for recruitment is long effective; the listed materials are required to provide electronic documentation, to be sent to my office e-mail: otalents@jnu.edu.cn

IV. Contact

Personnel department home page: <http://personal.jnu.edu.cn/>

Tel: 0086-20-85227283 (including fax), 0086-20-85223525

Contact: Mr. Tong, Mr. Liu

E-mail: otalents@jnu.edu.cn

Address: Human Resources Development and Management Service of Jinan University, No. 601, Huangpu Road West, Guangzhou.

Postal Code: 510632



Faculty Positions available at Hohai University, Nanjing, China

Hohai University invites applications for faculty positions at the assistant, associate, or full professor level in the area of engineering, science, economics, management, liberal arts, and law. Applicants should have a doctoral degree from a prestigious university. For the complete job announcements and directions on how to apply, visit: rsc.hhu.edu.cn or contact the Department of human resource at 86-25-83786205.

Hohai University, founded in 1915, wins its worldwide reputation on the research of Water Science & Civil Engineering & Environment Engineering. It is a National key university of China, and among the universities of the National "211 Project" and Innovation Bases of the National "985 Project". Hohai University aims to be a research oriented university.



上海交通大學
SHANGHAI JIAO TONG UNIVERSITY

Multiple Faculty Positions in Ultrafast Sciences / Electron Microscopy Shanghai Jiao Tong University, Shanghai, China

Shanghai Jiao Tong University is establishing a world-class research Center for Ultrafast Sciences in physics, chemistry, materials and biology with integration of both experimental and theoretical efforts. The Center will provide state-of-the-art ultrafast laser spectroscopy, electron diffraction and microscopy, and excellent multi-disciplinary environment for cutting-edge research.

The Center invites applications for a number of open-rank faculty positions to begin in the fall of 2016 (or earlier). Applicants should have a Ph.D. degree, preferably with postdoctoral experience in a closely related field, and a strong record of research accomplishments. Previous experience in electron microscopy and ultrafast sciences is favorable. The successful candidates will be expected to develop world-class research programs and teach classes at both undergraduate and graduate levels. The University will provide attractive annual salary, start-up fund, housing allowance and other benefits.

All applicants should send a cover letter, a curriculum vitae with a publication list, a research proposal (3-4 pages) and a statement of teaching interest in a single pdf file by Dec. 01, 2015 to Xiaoyan Li, Shanghai Jiao Tong University, Shanghai 200240, China through e-mail to: ultrafast@sjtu.edu.cn. Please also arrange three reference letters to be sent directly to the above e-mail address. Applications after the deadline could be reviewed until the positions are filled.

<http://join.sjtu.edu.cn/>

NMTDI Post-doctoral Position in Translational Cancer Research

A unique post-doctoral position in translational cancer research emerging from the partnership between the Northwestern Medicine Developmental Therapeutics Institute (NMTDI) and the Robert H. Lurie Comprehensive Cancer Center of Northwestern University.

The goal of this position is to advance the career of exceptional individuals committed to bridge basic research to clinical investigation and drug development. The selected scientist will be supported by a dynamic group of physician-scientists at NMTDI focused in transforming cancer care. A prolific network of ongoing and innovative collaborations coupled with the resources of a premier comprehensive cancer center accelerates clinically applicable scientific discoveries in an interdisciplinary and collegial working environment. The successful applicant will work in the basic science laboratory of physician scientist, Dr. Sarki Abdulkadir and be co-mentored by medical oncologists and clinical investigators, Drs. Benedito Carneiro and Francis Giles.

Individuals who are Ph.D. holders or physicians (M.D. or M.D./Ph.D.) following residency/fellowship training may apply by submitting CVs and names of three referees to:

Benedito Carneiro, MD
Northwestern Medicine Developmental Therapeutics Institute
Assistant Professor, Division of Hematology/Oncology
233 East Superior St, Olson Pavilion, 1st Floor
Chicago IL 60611
benedito.carneiro@northwestern.edu



The Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) is searching for a

Director

In accordance with the principles and mandate of the Max Planck Society, the MPI-CBG is seeking the scientific leaders of tomorrow and is interested in original and innovative research employing multi-scale and interdisciplinary approaches in the general area of molecular cell biology and developmental genetics with a particular focus on 'how cells form tissues'. Researchers early in their career who have already demonstrated the beginnings of a strong trajectory of originality and discovery are encouraged to apply. The MPI-CBG is particularly interested in recruiting outstanding female scientists. The successful candidate's research program will complement the research ongoing at the institute (see <http://www.mpi-cbg.de>).

Directors at Max Planck Institutes have unlimited appointments as members of the Max Planck Society and receive generous long-term support.

Please send your CV, publication list, a short description of research accomplishments and future plans to the current Managing Director of MPI-CBG at the address below. Applications will be considered upon receipt.

Wieland B. Huttner
Max Planck Institute of Molecular Cell Biology and Genetics
Pfotenhauerstr. 108
01307 Dresden, Germany
meder@mpi-cbg.de
+49 351 210 1500



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DTU Systems Biology. The successful candidate will be expected to lead an innovative research group and develop new research directions in the department. The candidate will be expected to securing funding for their research from Danish, European or other international funding agencies.

Application deadline: **5 November 2015**

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Protein Science

DTU Systems Biology. The successful candidate is expected to contribute through forefront research and innovation within at least one of the areas of our Protein Science section mentioned above and also to lead further developments of our engineering education within the area of Protein Science.

Application deadline: **5 November 2015**

PROFESSOR

Bacterial Physiology and Genetics

DTU Systems Biology. The position is available immediately and will carry responsibilities in both research and teaching. The applicant must have a strong research foundation in bacterial physiology, genetics and molecular biology and is expected to further strengthen these fundamental research areas.

Application deadline: **5 November 2015**

PROFESSOR

(with special responsibilities) Immunoinformatics and Machine-learning

DTU Systems Biology. The successful candidate is expected to contribute through forefront research and innovation to maintain this edge within Immunoinformatics of our Protein Science section and also to lead further developments of our engineering education within the general area of bioinformatics and machine learning.

Application deadline: **5 November 2015**

DTU is a technical university providing internationally leading research, education, innovation and public service. Our staff of 5,800 advance science and technology to create innovative solutions that meet the demands of society; and our 10,300 students are being

educated to address the technological challenges of the future. DTU is an independent academic university collaborating globally with business, industry, government, and public agencies.

Further details: career.dtu.dk



SCIENCE AND TECHNOLOGY AT A GLOBAL SCALE
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Cell Biology Feature

Issue date: December 4, 2015

Reserve ads by November 17

Ads accepted until November 30 on
a first-come, first-served basis



For recruitment in science, there's only one **Science**

Looking to hire cell biologists? This feature covers the growing trends in cell biology and the skills scientists need for a career in this field, such as biophysics, imaging techniques, and translational research. Advice from government, university, and company representatives for building a career in these expanding areas will draw scientists to your ad.

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CARNEGIE SCIENCE

POSTDOCTORAL FELLOWSHIPS

The Geophysical Laboratory, Carnegie Institution of Washington, invites applications for postdoctoral fellowships. The Geophysical Laboratory emphasizes interdisciplinary experimental and theoretical research in fields spanning geoscience, microbiology, chemistry, and physics. The Laboratory supports world-class facilities in high-pressure research; organic, stable isotope and biogeochemistry; mineral physics and petrology; and astrobiology. Please visit website: <https://jobs.carnegiescience.edu/jobs/carnegie-fellowships-for-the-geophysical-laboratory-3/> to view a list of required materials and application instructions. Also, see website: <http://www.gi.ciw.edu/> for a listing of personnel, current research interests, and major facilities.

Completed applications for Carnegie fellowships should be submitted by **December 1, 2015**.

The Geophysical Laboratory is located in Washington, DC, and is an Equal Opportunity Employer.

U.S. POSTAL SERVICE

Statement required by the Act of 12 August 1970, Section 3685, Title 39, United States Code, showing the ownership, management, and circulation of:

1-9. Science, Publication No. 0036-8075, is published weekly on Friday, except the last week in December, at 1200 New York Avenue, N.W., Washington, DC 20005. Date of filing: 22 September 2015. This is also the address of the publisher, the editor, and the managing editor, who are, respectively, Kent Anderson, Marcia McNutt, and Monica M. Bradford.

10. The owner is the American Association for the Advancement of Science, 1200 New York Avenue, N.W., Washington, DC 20005. Stockholders: None.

11. Known bondholders, mortgages, and other security holders owning or holding 1 percent or more of total amount of bonds, mortgages, or other securities: None.

12. The purpose, function, and nonprofit status of this organization and the exempt status for federal income tax purposes have not changed during the preceding 12 months.

13-15. The average number of copies of each issue during the preceding 12 months is (A) Total number of copies printed: 96,517; (B) Paid circulation: 78,562; (1) Paid/Requested outside-county mail subscriptions stated on form 3541: 66,772; (2) Paid/Requested in-county subscriptions stated on form 3541: 0; (3) Sales through dealers and carriers, street vendors, counter sales: 11,778. (4) Other classes mailed through USPS: 12; (C) Total paid circulation: 78,562; (D) Free distribution: samples, complimentary, and other free copies: 15,928; (1) Outside-county as stated on form 3541: 2,554; (2) In-county as stated on form 3541: 0; (3) Other classes mailed through the USPS: 2; (4) Free distribution outside of mail carrier or other means: 13,372; (E) Total free distribution: 15,928; (F) Total distribution: 94,490; (G) Copies not distributed: 2,027; (H) Total: 96,517; (I) Percent paid and/or Requested Circulation: 83.1%.

Actual number of copies of single issue (9/11/2015) published nearest to filing date are (A) Total number of copies printed: 81,200; (B) Paid circulation: 75,690; (1) Paid/Requested outside-county mail subscriptions stated on form 3541: 64,731; (2) Paid/Requested in-county subscriptions stated on form 3541: 0; (3) Sales through dealers and carriers, street vendors, counter sales: 10,947; (4) Other classes mailed through USPS: 12; (C) Total paid circulation: 75,690; (D) Free distribution: Samples, complimentary, and other free copies: 3,475; (1) Outside-county as stated on form 3541: 2,545; (2) In-county as stated on form 3541: 0; (3) Other classes mailed through the USPS: 2; (4) Free distribution outside of mail: Carrier or other means: 928; (E) Total free distribution: 3,475; (F) Total distribution: 79,165; (G) Copies not distributed: 2,035; (H) Total: 81,200; (I) Percent paid and/or Requested Circulation: 95.6%.

I certify that the statements made above are correct and complete. (signed) Kent Anderson, Publisher.



Creation of new junior research groups at Institut Pasteur

The Institut Pasteur has launched an international call for junior candidates wishing to establish new independent research groups in the cutting edge interdisciplinary environment of its campus in Paris, France.

The Institut Pasteur is a non-profit private foundation dedicated to fundamental, interdisciplinary research and to the translation of the knowledge to medicine and public health. Topics of interest include microbiology (bacteria, viruses, parasites and fungi) and infectious diseases, immunology, developmental biology and stem cells, neuroscience, genomics, structural biology, genetics and cancer.

Successful **junior candidates*** will be appointed with a permanent position, and as head of a group of 6 people. These groups will be created for a period of 5 years and may thereafter compete for a full research group.

Highly attractive packages to match the experience of the candidate will be provided, including institutional salaries (Principal investigator, technician, secretary, post-doctoral fellows), a substantial contribution to running costs and equipment, access to on campus state-of-the-art technology core facilities, as well as support for relocation expenses and administrative issues.

Applications shall be submitted online at: <https://aap3.voozanoo.net/register> and shall include:

1. A web form summarizing the application.
2. A complete application file using the template which can be downloaded from the submission website.

The template should be filled and uploaded as a single PDF file.

The deadline for submission of the applications is **December 2, 2015**, by 5:00 pm CET. Shortlisted applicants will be notified by mid-January 2016, and will be invited for interview to take place mid-February 2016. The final ranking will be established by the Pasteur Scientific Council during its March 2016 session.

Further information on the institute can be found at <http://www.pasteur.fr>

Contact: aap3@pasteur.fr

** Institut Pasteur is an Equal Opportunity Employer. Junior group leaders should be less than 8 years after PhD at the time of submission. Women are eligible up to 11 years after their PhD if they have one child and up to 14 years after their PhD if they have two or more children.*

MONTEREY BAY AQUARIUM RESEARCH INSTITUTE

2016 POSTDOCTORAL FELLOWSHIP PROGRAM

Applications for the postdoctoral fellowship program at the Monterey Bay Aquarium Research Institute (MBARI) are currently being accepted. MBARI is dedicated to the development of state-of-the-art instrumentation, systems, and methods for scientific research in the oceans. Ongoing programs in marine robotics, ocean physics, chemistry, geology, and biology as well as information management and ocean instrumentation research and development exist at MBARI. Located in Moss Landing, California at the head of Monterey Canyon, MBARI enjoys convenient access to diverse oceanographic environments. The institute operates research vessels equipped with remotely operated vehicles, autonomous underwater vehicles, and diverse oceanographic equipment. In addition, MBARI operates the MARS seafloor cabled observatory. MBARI is a non-profit oceanographic research institute supported by the David and Lucile Packard Foundation.

Offers will be made to candidates from the fields of biological, chemical, and physical oceanography; marine geology; and ocean engineering. Candidates must be awarded the Ph.D. degree prior to commencing the two-year appointment and start during the 2016 calendar year. Applicants are encouraged to communicate with potential research sponsors at MBARI for guidance on project feasibility, relevance to ongoing research projects, and resource availability (http://www.mbari.org/about/postdoc_mentors.htm).

Application deadline: Wednesday, December 9, 2015

Selected candidates will be contacted in early March 2016.

Application requirements:

1. Curriculum vitae
2. At least three professional letters of recommendation
3. Succinct statement of the applicant's doctoral research
4. Potential research goals at MBARI
5. Supplemental information online form (http://www.mbari.org/oed/jobs/forms/postdoc_form_2016.htm)

Address your application materials to:

MBARI, Human Resources Job code: Postdocs-2016
7700 Sandholdt Road, Moss Landing, CA 95039-9644

Submit by e-mail to: jobs_postdocs@mbari.org (preferred),
by mail, or fax to (831) 775-1620.

MBARI is an equal opportunity and affirmative action employer. MBARI considers all applicants for employment without regard to race, color, religion, sex, national origin, age, disability, or covered veteran status in accordance with applicable federal, state, and local laws. Competitive compensation and benefits package.



EOE • MBARI Welcomes Diversity

By Tal Polak

Help others—and help your career

As a Ph.D. student, I try to volunteer at every conference I attend. Initially, the motivation was purely monetary: Students who volunteer usually get discounted registration fees. However, during my first conference, I came to appreciate how important volunteering can be for networking. While welcoming people at the admission desk or offering speakers assistance, volunteers get to know a lot of scientists. During my second large conference, the 2013 International Congress for Conservation Biology (ICCB) in Baltimore, Maryland, I leveraged my volunteer position to create an even more rewarding networking opportunity.

I was volunteering at the front desk when I heard an announcement about a fundraising auction. The auction, which aimed to raise money to support the Society for Conservation Biology's chapters, was planned for the closing party. Participants were invited to provide auction items, such as signed books, paintings, and wine. This sparked an idea: You probably know the concept of having a bachelor auction, but, as we were at a scientific conference, why not have a scientist auction?

Conferences are a great venue for students to meet leading scientists in their field, but, in practice, such opportunities are rare. Typically, prominent scientists seem surrounded by people at all times, and students can be shy. So, my idea was to add 2-hour personal meetings with leading scientists to the list of items for auction.

The auction, however, was in 3 days, and I was just a student volunteer. Luckily, members of the conference planning committee had purple ribbons on their name tags, and so I started pitching my idea to anyone with a purple ribbon. Eventually, I reached the president of the society, who not only gave me his permission but also promised to participate as an auctioned scientist. Next, I had to convince other top researchers to take part. I started with my own supervisor, who agreed and referred me to other people he knew, and so on. Altogether, I convinced six scientists. In the process, I talked to 30 of the leading scientists in my field, who probably still remember my name, where I'm from, and what I do.

During the auction, I encouraged supervisors to bid on meetings that could help their students advance their careers or secure new collaborations for the lab. I prompted students interested in doing a postdoc with one



"I talked to 30 of the leading scientists in my field, who probably still remember my name."

of the leading scientists to bid on an opportunity to talk with them before sending a formal application. I promoted bids on scientist-authors by saying, "Why buy the book, when you can buy the author?" I finished the night exhausted but with all six scientists bid upon. The effort raised an extra \$300 for the chapters, which will serve to fund student awards. And most of the attendees left the event knowing me.

This year, I organized a similar event at the ICCB in Montpellier, France. While coveted 2-hour personal meetings were still offered, we decided to make the event more affordable to students and people from developing countries by replacing the scientist auction with a scientist raffle. We also ran the event earlier in the conference

so that the auctioned meetings could take place onsite.

Raising funds for students and providing them with opportunities to talk to leading scientists have proved hugely rewarding. I know of at least one new collaboration that has emerged from such meetings. And from the personal connections I made during the first auction event, I have formed collaborations with senior scientists and received invitations to travel overseas. So next time you go to a conference, offer yourself up as a volunteer and create your own good reason for all the scientists you approach to remember you. ■

Tal Polak is a fourth-year Ph.D. student in conservation biology at the University of Queensland, St. Lucia, in Australia and the Australian Research Council Centre of Excellence for Environmental Decisions. For more on life and careers, visit sciencecareers.org. Send your story to SciCareerEditor@aaas.org.